First Responders to Cataclysmic Upheaval: Earthquake–Driven Effects on Microalgae in the Avon-Heathcote Estuary, Christchurch, New Zealand.

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ABSTRACT

The Avon-Heathcote Estuary is of significant value to Christchurch due to its high productivity, biotic diversity, proximity to the city, and its cultural, recreational and aesthetic qualities. Nonetheless, it has been subjected to decades of degradation from sewage wastewater discharges and encroaching urban development. The result was a eutrophied estuary, high in nitrogen, affected by large blooms of nuisance macroalgae and covered by degraded sediments. In March 2010, treated wastewater was diverted from the estuary to a site 3 km offshore. This quickly reduced water nitrogen by 90% within the estuary and, within months, there was reduced production of macroalgae. However, a series of earthquakes beginning in September 2010 brought massive changes: tilting of the estuary, changes in channels and water flow, and a huge influx of liquefied sediments that covered up to 65% of the estuary floor. Water nitrogen increased due to damage to sewage infrastructure and the diversion pipeline being turned off. Together, these drastically altered the estuarine ecosystem. My study involves three laboratory and five *in situ* experiments that investigate the base of the food chain and responses of benthic microalgae to earthquake-driven sediment and nutrient changes. It was predicted that the new sediments would be coarser and less contaminated with organic matter and nutrients than the old sediments, would have decreased microalgal biomass, and would prevent invertebrate grazing and bioturbation activities. It was believed that microalgal biomass would become similar across new and old sediments types as the unstable new sediments were resuspended and distributed over the old sediments. Contact cores of the sediment were taken at three sites, across a eutrophication gradient, monthly from September 2011 to March 2012. Extracted chlorophyll *a* pigments showed that microalgal biomass was generally lower on new liquefied sediments compared to old sediments, although there was considerable site to site variation, with the highly eutrophic sites being the most affected by the emergence of the new sediments. Grazer experiments showed that invertebrates had both positive and negative site-specific effects on microalgal biomass depending on their identity. At one site, new sediments facilitated grazing by *Amphibola crenata*, whereas at another site, new sediments did not alter the direct and indirect effects of invertebrates (*Nicon aestuariensis*, *Macropthalmus hirtipes*, and *A. crenata*) on microalgae. From nutrient addition experiments it was clear that benthic microalgae were able to use nutrients from within both old and new sediments equally. This implied that microalgae were reducing legacy nutrients in both sediments, and that they are

an important buffer against eutrophication. Therefore, in tandem with the wastewater diversion, they could underpin much of the recovery of the estuary. Overall, the new sediments were less favourable for benthic microalgal growth and recolonisation, but were less contaminated than old sediments at highly eutrophic sites. Because the new sediments were less contaminated than the old sediments, they could help return the estuary to a non-eutrophic state. However, if the new sediments, which are less favourable for microalgal growth, disperse over the old sediments at highly eutrophic sites, they could become contaminated and interfere with estuarine recovery. Therefore, recovery of microalgal communities and the estuary was expected to be generally long, but variable and site-specific, with the least eutrophic sites recovering quickly, and the most eutrophic sites taking years to return to a pre-earthquake and non-eutrophied state.

CHAPTER 1

Introduction

This thesis focuses on benthic microalgal (BMA) dynamics in a highly disturbed estuary. Much of the work and ensuing discussion relates to large changes brought about by a series of earthquakes and the responses of the benthic community to resulting nutrient and sediment loads. Before the earthquakes, the estuary was in a highly eutrophic condition, brought on by over 100 years of urban development, changes in land use practices in the catchment, and the outfall from a city sewage treatment plant (Owen 1992). After wastewater was diverted to an ocean outfall the estuary showed signs of recovery, but this recovery was disrupted by several earthquakes (Zeldis et al. 2011). It was therefore important to examine the responses of BMA to the high sediment and nutrient loads that accompanied the earthquakes to help determine the ways in which the estuary had been affected and how the trajectory of recovery from eutrophication had been altered.

Benthic microalgae are small unicellular photosynthetic protists associated with bottom sediments in environments such as estuaries (Graham & Wilcox 2000). They have the potential to sequester nutrients in sediments (Piehler et al. 2010), and this function has become increasingly important because estuaries have proved to be convenient repositories for urban waste (McLusky 1971). Some of the most important discharges include organic matter, toxic chemicals and sewage (Barnes 1984), with large additions of sewage derived nutrients leading to high amounts of primary production and eutrophication (Mann 2000). Eutrophication can cause algal blooms to occur, blocking sunlight to the environment below (Bricker et al. 1999). As these algal blooms decay, oxygen that was once available to fish and other animals is used up in the water column, leading to mortalities of many species (Bricker et al. 1999). With the resulting degraded water quality, the use of estuaries for recreational activities such as fishing and boating can be adversely affected and dangers to human health can occur if shellfish contaminated with algal toxins are consumed (Bricker et al. 1999). As BMA have the potential to sequester nutrients in the sediments, they can reduce the potential

for algal blooms in the water column (Piehler et al. 2010), effectively acting as buffers against eutrophication.

The structure of BMA communities is regulated by interactions between bottom-up, top-down and disturbance events (Hagerthey et al. 2002). Disturbance events can be defined as any uncommon, relatively discrete event that causes changes in the physical environment (Pickett & White 1985) or natural communities (Sousa 1984), moving them away from static or equilibrium conditions (Sousa 1984). Although BMA live in environments subjected to violent fluctuations, they can completely disappear in the event of catastrophic disturbances such as earthquakes and floods (Cooper 1996). This is partially because each microalgal species has fairly precise requirements for growth and survival (Cooper 1996). Populations can recover from intermediate disturbances via succession, but catastrophic disturbances are capable of dramatically reducing biodiversity (Montagna et al. 1998, Lohrer et al. 2010). A change in BMA community structure could lead to changes in the way estuarine communities function, through impacts on primary productivity (Piehler et al. 2010). The integrity of benthic ecosystems depends on the ability of their communities to recover from disturbance (Norkko et al. 2010) and a decrease in ecosystem functioning can lead to a loss of resilience and the inability to recovery from future disturbance events (Lohrer et al. 2010).

The importance of many environmental factors in regulating BMA in sediments is not completely understood (Davis & Lee 1983), nor are changes in functioning after disturbance and during recovery (Lohrer et al. 2010). Disturbance-recovery experiments can shed light on processes, feedbacks and thresholds that are important for ecosystem resilience (Thrush et al. 2009). Because of the functions that BMA perform in the ecosystem, understanding the factors that control the structure and function of the intertidal flat BMA, as well as quantifying their biomass and productivity, may be particularly important for predicting the success of communities in an environment categorised by significant stressors (Piehler et al. 2010).

1.1 THE ESTUARINE ENVIRONMENT: A REVIEW AND INTRODUCTION TO THE AVON-HEATHCOTE ESTUARY

1.1.1 What is an estuary?

Estuaries are usually formed by the drowning of river valleys, or by a rise in sea level that causes submergence of parts of a coast (McLusky 1971, Little 2000, Mann 2000). Some develop in fjords, while others are formed by the deposition of sand bars and shingle spits that trap freshwater discharge, but allow access from the sea (McLusky 1971, Little 2000, Mann 2000). Although they can be defined in many ways, Pritchard (1967) gave the most comprehensive definition when he stated that an estuary is a semi-enclosed coastal body of water that has a free connection to the sea. He extrapolated that it is also a place where seawater is measurably diluted with freshwater derived from land drainage.

The Avon-Heathcote Estuary (43.5470°S, 172.7230°E) is a triangular shaped, semi-enclosed body of water (Figure 1.1) that formed about 450 years ago when sediment deposition built up to create the southern spit, and seawater pooled behind it (Owen 1992). The estuary borders the eastern suburbs of Christchurch, New Zealand and is approximately eight square kilometres in area, with nearly 11 million cubic metres of seawater flowing in and out with each tidal cycle (Owen 1992). Unlike many estuaries worldwide, the Avon-Heathcote Estuary (AHE) is shallow (1.4 m mean depth at high water on spring tides), microtidal (spring tide range = 2.1 m), mostly intertidal (85% of the area is made up of intertidal mudflats) and, except for channels, is largely drained on low tides (Knox & Kilner 1973, Griffin & Thomson 1992).

The Avon River enters the estuary in the northern corner, the Heathcote River in the west and seawater in the east (Figure 1.1; Davis & Lee 1983). With influences from both fresh and saltwater, this estuary becomes a unique intermediate between ocean, terrestrial and freshwater ecosystems (McLusky 1971). Being influenced by many different ecosystems, estuaries are prone to environmental changes and fluctuations in both physical and biological factors through time and space (McLusky 1971, Knox & Kilner 1973).

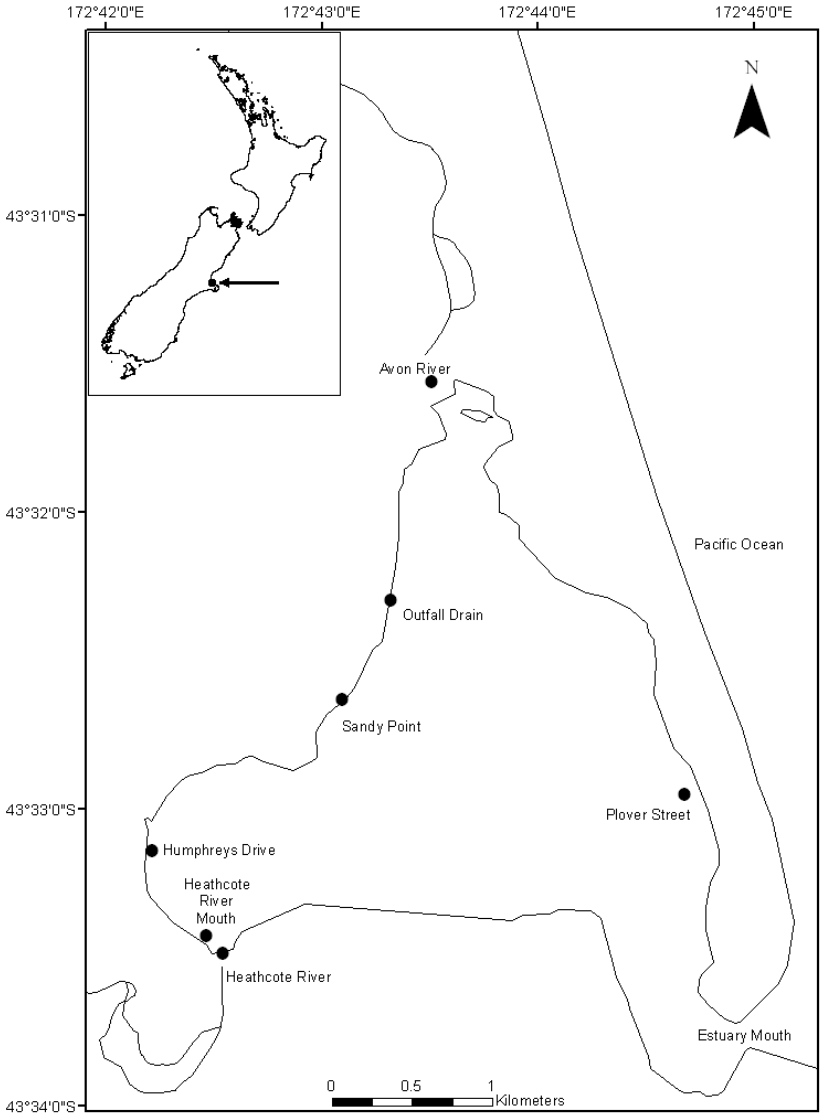

Figure 1.1: Map showing the location of the Avon-Heathcote Estuary within New Zealand, sites used for this study (Humphreys Drive, Plover Street, and the Heathcote River Mouth), and other areas of interest (Avon and Heathcote Rivers, the outfall drain and Sandy Point).

1.1.2 Physical Characteristics

The following attributes of the AHE pertain to the time period leading up to the major earthquakes that began in September 2010. The effects of the earthquakes on the current physical and biological characteristics of the estuary are still being ascertained (as at September 2012), and will be addressed in the following chapters.

1.1.2.1 Salinity

With the influence of riverine freshwater and oceanic saltwater, the salinity of estuarine water can vary from largely freshwater to full strength seawater or above, and it fluctuates according to site, topography, position of inlets, season and tidal stage (Barnes 1984, Little 2000). In the AHE, there is generally a decreasing salinity gradient from the mouth of the estuary towards the Avon and Heathcote Rivers (Owen 1992), with salinity varying by site and ranging from 0.8-29.3‰ at low tide and from 10.2-33.7‰ at high tide (Bolton-Ritchie 2008). Tides are semi-diurnal and can push saline water approximately 10 km up the Avon and Heathcote Rivers (Knox & Kilner 1973). Even though the estuary is largely drained during the ebbing tides, an estimated 44% of the inflowing freshwater returns with the following tide, suggesting that freshwater has a significant influence within the estuary (Knox and Kilner 1973).

Because freshwater is less dense than saltwater, the estuarine water column can be stratified, but may also be partially mixed or homogenous, depending on the influence of currents and river flow (Little 2000). In the AHE, the shallow depth, strong winds and strong tidal action of the estuary interact to create considerable vertical mixing in the water column, but salinity profiles indicate the presence of some stratification at some sites (Owen 1992). Stratification in the water column can lead to flocculation of sediment and nutrients, when the sediments sink from river water into the saline water below (Owen 1992). During the incoming tide, sediments are deposited in the estuary by the net landward flow of saline water from the sea (Mann 2000).

1.1.2.2 Sediments

Prior to rivers reaching the estuary they have already deposited their larger, heavier suspended sediments upstream (Barnes 1984). Most sediments in motion in estuaries are, therefore, small silt particles (4-64 µm diameter) carried in suspension (Barnes 1984). Sedimentation of these particles occurs where water currents slacken and drop their sediment, which frequently occurs in the central region of the estuary, creating mud flats (McLusky 1971). Suspended mud can also be brought in with tides and will often remain because of wave sheltered conditions, with an average of 2 mm of sediment being accumulated in some estuaries annually (Barnes 1984).

Most of the AHE is composed of soft substratum, with sediments above the low water mark generally being of larger size than those closer to the channels that run from the river inputs to the estuary mouth (Owen 1992). There is a gradient from mud (silt and clay) at the river entrances, to sand near the seaward mouth of the estuary (Owen 1992, Bolton-Ritchie 2011a), with particularly fine sedimented areas also occurring near the old city outfall drain (Figure 1.1; Knox and Kilner 1973). Because approximately 80% of the fine sediment that enters the estuary each year is deposited in the Avon and Heathcote depositories, these sites are muddier than elsewhere in the estuary (Deely 1993).

1.1.3 Biological Characteristics

The biota of the estuary must be able to cope with the fluctuations in the physical factors listed above and must withstand regular cover and exposure by the semi-diurnal tides (Owen 1992). Only a few species have adapted to these changing environments and many may be living at the limits of their tolerance to one or more physical factors (Jones 1983, Owen 1992). These abiotic factors include things such as salinity, sediment particle composition and exposure time and they are responsible for driving the distribution and abundance of biota within estuaries (Owen 1992, Bressington 2003).

1.1.3.1. Macrofauna

Estuarine macrofauna comprise two ecologically distinct groups: semi-sessile residents (mainly invertebrates) and mobile species that forage widely at high or low tide (fish and birds, respectively; Barnes 1984). The AHE is a natural habitat for a rich and diverse community of birds (Owen 1992) and fish (James 1999). Over 100 species of bird have been recorded, including geese, ducks, Pukeko, spoonbills and cormorants (Owen 1992). The estuary is internationally recognised as an important wetland for birds and contains 5% of the South Island pied oystercatcher (*Haematopus ostralegus finschi*) population (Owen 1992). Thirty four species of fish have been documented in the estuary (James 1999), with sand flounder (*Rhombosolea plebeia*), globefish (*Contusus richei*) and yellow eyed mullet (*Aldrichetta forsteri*) being among the most common (Knox & Kilner 1973).

Most invertebrates in the AHE can be characterised as burrowers, which are able to escape predators, salinity changes and desiccation as the tide rises and falls (Jones 1983). These burrowing invertebrates can be further classified according to their trophic groups: browsers

(species that move and graze on the sediment surface for detritus and microalgae), filter feeders (species that use their ciliated feeding apparatus to feed on the particles in the water column), and deposit feeders (species that ingest a mixture of inorganic and organic matter, but remove only the organic matter; Mann 2000). The most abundant taxa are polychaete worms (*Glycera Americana*), cockles (*Austrovenus stutchburyi*), hairy handed mud crabs (*Hemigrapsus crenulatus*) and mudflat snails (*Amphibola crenata*) (Knox & Kilner 1973, Owen 1992, Bressington 2003). *Amphibola crenata* is a highly abundant air breathing deposit feeder, eating bacteria and benthic microalgae, and leaving behind a slimy secretion on which bacteria feed (Juniper 1982, 1987, Griffin & Thomson 1992).

1.1.3.2 Macrophytes

Macrophytes are important to the functioning of the estuary because they absorb dissolved nutrients from the water column and provide a large amount of organic carbon to the sediments for use by bacterial decomposers (Figueiredo da Silva et al. 2009). The vegetation of the AHE is dominated by the green algal complex, *Ulva* spp. (sea lettuce and the form previously known as *Enteromorpha*) and the red alga, *Gracilaria chilensis* (Bolton-Ritchie 2011a), with smaller patches of the sea grass, *Zostera muelleri* (Steffensen & McGregor 1976). *Ulva* covers approximately 80% of the floor of the estuary at some sites (Bolton-Ritchie 2011a). The seasonal proliferation of macroalgae has led to an accumulation of decaying, foul smelling, algal masses along the shore line (Bolton-Ritchie & Main 2005), particularly of *Ulva*, which are readily able to utilise the high concentrations of nutrients (Rivers & Peckol 1995).

1.1.3.3 Benthic microalgae

In the AHE, pennate diatoms (division Chrysophyta), cyanophytes (cyanobacteria or blue-green algae) and euglenophytes (euglenoids) are the dominant BMA assemblages and they are sometimes visible as brown, green or golden brown films (Knox & Kilner 1973). BMA density in shallow water sediments can vastly exceed that of planktonic microalgae (Vilbaste et al. 2000). About 70% of BMA are found in the top millimetre of sediments during emersion (Longphuirt et al. 2009), with cell densities of up to a million per square centimetre (Palmer & Round 1965). The advantage of occupying the uppermost layers of the sediment is that there is a great amount of light available for photosynthesis (Pinckney & Zingmark 1993). Light declines rapidly in surface sediments, making photosynthesis and survival nearly impossible below the first few millimetres (Pinckney & Zingmark 1993).

Photosynthesis by BMA can contribute up to one third of the total primary production of estuarine mudflats (Revsbech & Jorgensen 1983), providing a source of readily usable organic matter to other organisms (Little 2000). BMA provide the bulk of food for bottom dwelling invertebrates, and without them the invertebrates and larger species that feed on them would perish (Cooper 1996). Due to this, and to their ability to feed on nutrients from both overlying water (Round 1981) and porewater (Sundback & Graneli 1988), BMA play a critical role as nutrient cyclers and connectors of the benthic-pelagic food chain (Rizzo 1990, Peletier 1996). The organic and inorganic material incorporated into algae as they grow can be recycled through the water column after their death (Graham & Wilcox 2000) and some of this organic matter gets buried in the sediments through mixing by invertebrates in a process known as bioturbation (Mann 2000).

BMA biomass and production are primarily affected by substrate movement, grain size, nutrient levels, currents and physic-chemical gradients in oxygen, and temperature (Steffensen 1974, McClatchie et al. 1982, Juniper 1987, Sundback & Graneli 1988, Cane 1996, Hayward 1997, Underwood et al. 1998). Even a slight change in sediment grain size or in the balance of nutrients can be reflected in the changing composition, abundance and biomass of BMA communities (Cooper 1996, Grinham et al. 2011). In terms of biological influences, BMA are subject to both competition with other algal groups, such as phytoplankton (Fong et al. 1993), and grazing pressure from invertebrates (Hillebrand & Kahlert 2002).

Microalgae are not entirely passive within the environmental extremes they experience. Many diatoms and flagellates are motile and able to migrate within the sediment to avoid less optimal conditions, such as high temperatures, or to gain access to favourable conditions, such as light for photosynthesis (Palmer & Round 1965, Little 2000, Mann 2000). The migration occurs within the top 1-2 mm of sediment and follows a tidal and diurnal rhythm (Little 2000) that can persist in laboratory conditions for up to a month (Palmer & Round 1965).

1.2 THE AVON-HEATHCOTE ESTUARY: A VALUABLE, BUT CONTAMINATED, RESOURCE

The AHE is an important natural asset to Canterbury, not only because of its high productivity and biotic diversity (Knox & Kilner 1973, Owen 1992), but also because it is used frequently for a variety of water based (surfing, sailing) and land based (bird watching, picnicking) recreational activities (Bolton-Ritchie & Main 2005). Because it is easily accessible and so close to a city, there is high aesthetic value, with many walkways and reserves around its perimeter (Owen 1992).

Although it has always been important to the city of Christchurch, the estuary has been sequentially degraded over the past 150 years (Knox & Kilner 1973). Considerable urban development has occurred around it, with attendant draining of freshwater wetlands and burning and logging of coastal forests (Knox & Kilner 1973, Owen 1992). In response to urbanisation in, a sewage farm was constructed at the future site of the oxidation ponds in 1882, where treatment of incoming wastewater was accomplished by settling ponds and spreading of effluent over paddocks (Bolton-Ritchie & Main 2005). From the paddocks, the wastewater percolated through the sand and into the estuary (Bolton-Ritchie & Main 2005). Settling ponds were later replaced by septic tanks, and then by the combination of settling tanks, trickling filters and oxidation ponds at the Christchurch City Council Wastewater Treatment Plant in 1962 (Bolton-Ritchie & Main 2005). The treatment plant underwent improvements in 1971, 1978, 1996 and 2004 (Bolton-Ritchie & Main 2005), so that sewage eventually went through three treatment stages, with the main treatment plant removing almost all solids and dissolved contaminants through screens and filters, and the oxidation ponds reducing 99.99% of pathogens through biological oxidation and UV light exposure (Miller et al. 2004). The effluent was then discharged through an outfall drain on the western side of the estuary, as it had been since the oxidation ponds were commissioned in 1962 (Miller et al. 2004, Bolton-Ritchie & Main 2005).

Despite these efforts, there were still large amounts of contaminants in the estuary (Owen 1992, Bolton-Ritchie & Main 2005); the mean volume of wastewater discharged from the oxidation ponds in 2001 was 160 000 m^3/day (Bolton-Ritchie & Main 2005) and nearly half of the effluent leaving the estuary returned with the incoming tide on the next flood tide (Knox & Kilner 1973). This gave rise to high levels of heavy metals, toxic organics, contaminated sediments, bacterial pathogens, ammonia-nitrogen, total nitrogen and

phosphorus, which increased in the discharged water as Christchurch grew (Knox & Kilner 1973, Owen 1992, Miller et al. 2004). Eutrophic conditions resulted in many areas, having high levels of nitrogen, organic content and phosphorous, especially in the silt dominated areas of the estuary (Bolton-Ritchie & Main 2005). The greatest concentrations of nitrate and nitrite were found close to the river mouths, whereas high concentrations of ammonium and phosphorous occurred close to the outfall from the oxidation ponds (Bolton-Ritchie & Main 2005).

The estuarine ecosystem has become greatly driven by the accumulated sediments, organic matter and high loads of nutrients resulting from over 150 years of sewage, or its byproducts, entering the estuary daily (Knox & Kilner 1973). The nutrient concentrations in the AHE are capable of producing adverse biological effects (Bolton-Ritchie 2008). At least until the wastewater diversion in March 2010, the estuary contained high abundances of sewage fungus (Cameron 1970), and was experiencing large blooms of microalgae and nuisance macroalgae (mostly *Ulva* sp.; Knox and Kilner 1973). Because of effects on the microbial community, there was a high oxygen demand on the interstitial water occupying the space between the sediments (Owen 1992). Removed oxygen cannot be quickly replaced because of the reduced lability of porewater and so an anoxic layer results, usually below the top 5-10 mm of the sediments (Barnes 1984). Variable but small amounts of oxygen are available above the anoxic layer (Barnes 1984). Many fish, benthic microalgae and invertebrates have specific oxygen requirements, so severe eutrophication can lead to a reduction of species diversity and a loss of production (McLusky 1971, Graham & Wilcox 2000, Mann 2000). Many estuary based recreational activities, such as swimming and shellfish gathering, have also been greatly affected (Owen 1992). As a solution to all of these issues, the creation of a 3 km long ocean outfall pipeline was proposed in 2004, which was anticipated to be operational by the end of 2009 (Miller et al. 2004).

1.3 WASTEWATER DIVERSION: AN ATTEMPT TO CLEAN UP THE ESTUARY

The discharging of wastewater into the estuary stopped in March 2010, when wastewater was finally diverted to the ocean outfall off the New Brighton coastline (Bolton-Ritchie 2011b). To determine the effects of the diversion in terms of ecosystem health and to track the recovery of the estuary, baseline sampling was done before the pipeline was operational. The University of Canterbury, in conjunction with NIWA, accomplished two major sampling

campaigns, along with monthly sampling at some sites for several months before the diversion took place. During the campaigns, nutrient and oxygen flux experiments were conducted on the sediment porewater, sediment chemistry was analysed, microalgae biomass and photosynthetic rates were determined, and benthic invertebrates were surveyed (joint programme of UC and NIWA, unpublished data).

After the oceanic outfall pipeline opened in March 2010, sampling continued and signs of recovery were apparent within the first six months. Macroalgal chlorophyll content reduced by about 50% (N Barr, NIWA, pers. comm) and water column nutrients declined by 90% (Bolton-Ritchie 2011b). It was predicted that the improvement in water quality would be the initial trigger for estuarine improvement, but these changes did not have time to track through the ecosystem before a series of earthquakes altered the estuary.

1.4 CHRISTCHURH'S EARTHQUAKES

New Zealand sits on the boundary between the Australian and Pacific Plates, which constantly grind into each other to produce earthquakes (Geonet 2011). From September 4th 2010 to January 10th 2012 there were 3149 earthquakes and aftershocks in the Canterbury region that were of magnitude 3.0 or greater (Geonet 2011).

In September 2010 a magnitude 7.1 earthquake occurred 40 km west of Christchurch city, near the town of Darfield (Geonet 2011). As a result, there was damage to the city's infrastructure and sewer pipelines and large amounts of raw effluent were discharged into the rivers that flow into the estuary for two months (Bolton-Ritchie 2011a). This earthquake initiated three other significant aftershocks that were also close to Christchurch (Geonet 2011). The first major aftershock, a magnitude 6.3, occurred on February 22nd 2011 within 10 km of Christchurch and resulted in much of the city of Christchurch being destroyed (Geonet 2011), tilting of the estuary floor (with the northern portions subsiding and the southern portions rising by 0.2-0.5 m), and massive liquefaction throughout the region, including in the estuary (Figure 1.2; Measures et al. 2011).

Figure 1.2: Mounds of new sediment caused by liquefaction during the February 2011 earthquake at (a) Plover Street and the (b) Heathcote River Mouth.

Liquefaction is the process that results in soil suddenly losing its strength and structure, most frequently as a result of ground shaking during earthquakes (Youd et al. 2001). Rapid shaking causes sediment grains to compress the porewater between them (Youd et al. 2001). The water increases in pressure, which reduces the contact forces between soil particles, reducing

their ability to maintain contact and weakening them (Morris 1983). During large earthquakes, the higher porewater pressures cause deeper liquefied sand and water to move upward to the ground surface, creating sand volcanoes and making the soil appear as though it flows (Youd et al. 2001). This occurs mostly in saturated, loose sandy soils, which are completely surrounded by water and not able to bind as well as clay particles (Morris 1983). In the case of the AHE, liquefied sediments were propelled to the surface in a volcano like fashion, creating mounds of new sediment that covered up to 65% of the estuary floor in new sediments (Zeldis et al. 2011). These were initially pristine, containing no nutrients or organic matter, unlike the old surface sediments of the estuary (Zeldis et al. 2011). Core samples showed that old sediments were initially buried as deep as 18 cm from the new surface sediments (Zeldis et al. 2011). These volcanoes of sediment gradually dissipated through time (Zeldis et al. 2011), although several new, smaller liquefying events occurred throughout 2011.

Liquefaction, along with tilting of the estuary floor, changed channels, tidal flows, tidal position of some sites, and caused tidal inundation of areas along the northern margin of the estuary (Measures et al. 2011). There was also further damage to sewer pipes leading into the ocean outfall pipeline, which was, consequently, turned off until November 2011 (Rutherford & Hudson 2011). Damaged sewer pipes resulted in a large influx of raw effluent, dumping into the estuary at a rate of 40-60 million litres per day (Rutherford & Hudson 2011) and water nitrogen levels increased (Bolton-Ritchie 2011b).

The diversion **s**ampling campaigns, involving the University of Canterbury and NIWA, continued throughout the earthquakes in order to determine the immediate changes imparted on the estuary. Baseline data collected in anticipation of the ocean outfall served as a good comparison.

1.4.1 Ecological consequences of earthquakes in the Avon-Heathcote Estuary

The earthquakes that caused liquefaction in the estuary were a series of disturbance events that interrupted the post-diversion recovery trajectory (Zeldis et al. 2011). The addition of sediments and nutrients during these events has the potential to drastically alter the estuarine ecosystem, starting at the base of the food chain with the microalgae. Changes in nutrient

levels may cause changes in algal community composition and effect production, thereby affecting organic carbon input into the rest of the food chain (Cane 1996, Graham & Wilcox 2000).

The arrival of the more pristine sediments and biotic changes certainly altered the estuary but may have helped facilitate its recovery from a eutrophied state (Zeldis et al. 2011). With the old contaminated surface being buried in many places, and the anoxic layer potentially being deeper in the new sediments, the estuary may become a cleaner environment qicker than projected before the earthquake. However, if the sediments have provided an unfavourable environment for the microalgae then there may be a decrease in production and nutrient cycling, with detrimental effects on the estuary's recovery.

1.5 JUSTIFICATION

The estuary is of great importance to the people of Christchurch, so it is not surprising that the diversion project had considerable publicity and a large amount of interest from the public. The Christchurch earthquakes completely interrupted the recovery process of the estuary after the wastewater diversion, and management will now require that the new physical and biological interactions are understood before the effects of wastewater diversion on estuary remediation can be delineated and understood. Disturbance-recovery experiments may assist in management decisions because they can shed light on processes, feedbacks and thresholds that are important for ecosystem resilience (Thrush et al. 2009).

Microalgae can contribute up to 70% of the organic carbon to an estuary (Little 2000), so it is crucial to quantify their biomass and productivity. Their aggregation and spatial variability can provide information on the mechanisms structuring the population, since these patterns are altered by both abiotic and biotic factors (Thrush 1991). Furthermore, since BMA have access to nutrients that have accumulated in the sediment, they may have the ability to change the speed of recovery once the wastewater is diverted again and overlying water nutrients decrease. They appear to be responding to the new sediments already, but the trajectories of these responses are not clear. It is important to be able to track these trajectories to be able to understand how past and future earthquakes will alter the ecosystem, and how the environment will respond once the ocean outfall pipeline is functional again.

1.5.1 Project aims

The goal of this project was to examine how liquefaction has affected microalgal colonisation, distribution, biomass and production, as the new, less contaminated, sediments (Zeldis et al. 2011) respond to a heavy influx of nitrogen, primarily in the form of ammonium. The spatial and temporal biomass and distribution patterns of BMA were monitored at three sites within the AHE. The relationships between nutrients, sediment grain size, sediment organic content, macroinvertebrate grazers, macroalgal competitors and the observed distributions of BMA were experimentally tested in a series of field and laboratory based experiments using new and old sediments.

CHAPTER 2

The effects of earthquake-driven sediment change on the spatial and temporal distribution and abundance of benthic microalgae

2.1 INTRODUCTION

High amounts of sediments are delivered to estuaries naturally, but anthropogenic activities such as land development, deforestation, farming and urbanisation greatly enhance sediment loading (Thrush et al. 2003). Because benthic microalgal (BMA) communities live at the sediment-water interface, they are particularly subject to sharp environmental changes, such as those associated with the quantity and/or type of sediment input into an estuary (MacIntyre & Cullen 1996). Sediment deposition can alter the distribution and abundance of BMA (Larson & Sundback 2012) not only because it smothers the surface and inhibits light penetration, but also because different taxa may be associated with different sediment grain sizes (Jesus et al. 2009, Grinham et al. 2011). Sediment grain size is one of the most important environmental variables that affect BMA, through changes in community composition, biomass, distribution and abundance (Pomeroy 1959, Brotas et al. 1995, Cartaxana et al. 2006, Brito et al. 2009, Jesus et al. 2009, Grinham et al. 2011). The size of sediment grains can alter the cohesive properties of the substrate (Bressington 2003, Montserrat et al. 2008), as well as the amounts of nutrients (Brito et al. 2009) and organic content (Morgans 1969) that are contained within.

If the deposited sediment layer is thin, the impacts on BMA can be low or benign because some BMA are capable of small scale migration (about 0.3 mm/h) into more favourable areas (Larson & Sundback 2012). However, the massive liquefaction events that occurred as a result of the Christchurch earthquakes deposited coarse sediment "mounds" that averaged between seven and thirteen metres in perimeter, four to eighteen centimetres in height and covered up to 65% of the estuary floor (Zeldis et al. 2011). Initial investigations showed that the new sediment mounds contained a larger proportion of sandy sediments than old sediments (> 63µm diameter; Zeldis et al 2011). Such changes in grain size could lead to

large changes in BMA community composition, biomass and distribution across sediment types (Amspoker & McIntire 1978, Cartaxana et al. 2006, Brito et al. 2009, Jesus et al. 2009). New sediments also contained up to 70% less organic content than the old sediments. Organic content is also capable of influencing BMA communities (Underwood & Provot 2000); although it provides a source of energy for BMA, its decomposition to ammonium via microbial mineralisation places a high oxygen demand on porewater and increases the amount of heterotrophic bacteria (Barnes 1984, Owen 1992), resulting in a less favourable, anoxic environment for BMA (Bolam et al. 2000).

As large contributors to primary production in estuaries and the main drivers in nutrient cycling, BMA substantially enhance the resilience of estuaries and contribute to the rapid recovery of important ecosystem functions (Larson & Sundback 2012). Variability in taxonomic composition could alter recovery after disturbances because certain groups, such as diatoms (division Chrysophyta), are highly resistant to several stressors that accompany eutrophication, such as hypoxia, hydrogen sulphide and toxicants (Larson & Sundback 2012). Compositional changes can also affect sediment stability, nutrient cycling and trophic dynamics (Janousek et al. 2007), because macroinvertebrates are capable of selectively ingesting BMA species (Buffan-Dubau et al. 1996, Hagerthey et al. 2002). Therefore, increased knowledge of BMA sensitivity to the new sediments, and their patterns of resilience and recovery, is necessary for the effective management of the Avon-Heathcote Estuary (AHE). Rates and patterns of recovery may be site-specific due to interactions with the physical characteristics of a site, and can also depend on the timing of the disturbance in relation to the season (Norkko et al. 2010).

In light of the importance of BMA in estuarine recovery, and the known detrimental effects of sedimentation on BMA, it is surprising that there have been so few studies that have considered the effects of sedimentation on BMA populations over extended spatial and temporal periods (Jesus et al. 2009, Larson & Sundback 2012). While Steffensen (1974), McClatchie et al. (1982), and Cane (1996) investigated the response of BMA species to other environmental factors in the AHE, only Wardle (2009) assesed at the biomass and composition of BMA communities in response to environmental factors across temporal, spatial and estuary wide scales. In this chapter, the distribution, abundance and community composition of BMA were compared between the new and old sediments at three sites in the AHE. The following questions were addressed:

(1) Do new and old sediments differ in their physical properties (such as grain size and organic matter)?
(2) Do BMA biomass, distribution and taxonomic composition differ across the new and old sediments?
(3) Are any changes in the distribution and abundance of BMA driven by changes in sediment grain size and organic content in the sediment?
(4) Over what period of time does recolonisation of BMA occur on the new sediments? How long will it take for BMA biomass and composition to recover?

The expectations were that new sediments would be composed of larger grain sizes and would have a lower percentage of organic content, as per Zeldis et al (2011). BMA biomass and abundance would be greater in areas with fine sediments and high organic content and would, therefore, be lower in the new sediments. The reduced organic content of the new sediments would favour a shift towards Cyanophyta in these areas because cyanobacteria are able to fix nitrogen gas to meet their nutrient requirements when nutrients are limiting (Graham & Wilcox 2000). They would, therefore, outcompete members from the other divisions. The greatest changes in BMA biomass and composition across sediment types would be expected to be seen at Humphreys Drive and the Heathcote River Mouth, which naturally contain more fine sediments than the Plover Street site (Knox & Kilner 1973). Over time, the BMA biomass and composition would become similar across new and old sediments as sediments from the less stable mounds were resuspended by wave action and distributed over old sediments. The result would be that areas that contained new sediment mounds and the areas that contained old sediments would eventually have similar grain sizes and organic content and would support similar BMA biomass and relative abundances. Due to the rate at which new sediment mounds appeared to be dispersing, this process was predicted to take approximately three months.

2.2 METHODOLOGY

2.2.1 Study sites

Three sites of differing physical and biological characteristics were chosen for this study to represent the spatial variability of the AHE. The Heathcote River Mouth site was chosen because of its close proximity to the Heathcote River and the resulting influence of

freshwater and sediment deposition on the ecology of this site. Humphreys Drive was included because it is one of the most eutrophic sites in the estuary, and Plover Street was chosen because it is largely influenced by seawater and is one of the least eutrophic. All sites contained new sediment mounds, were completely exposed during low tide, and fully submerged during high tide.

The Heathcote River Mouth (Figure 1.2b) has a shallow, evenly sloping intertidal region that extends down to the low tide channel of the Heathcote River. This site contains only two shore heights, low and mid shore, because a man made sea wall separates the site from a nearby road and prevents the presence of a true high tide area. The close proximity of this site to the Heathcote River means that it is influenced by inputs of nutrients that come from run off in the surrounding catchment areas (Knox & Kilner 1973). River water dilutes the tides so that this site has a lower salinity (1.2-16 ppt range during a tidal cycle; Bolton-Ritchie 2011b) than the other two sites, and remains fairly constant throughout the year (Bressington 2003). Although there are BMA at this site, they are not always visible. When it is possible to see BMA mats, they appear orange and brown in colour and can be seen on the margins of the new sediment mounds. The rarity of visible BMA mats could be due to the lesser tidal influence at this site, as a result of the sea wall, which results in less exposure time to photosynthetic light intensities for the BMA (Wardle 2009). This site regularly supports a dense population of *Gracilaria chilensis* (around 75% cover all year; Bressington 2003), and the decay of this algae contributes to the low dissolved oxygen levels found here (70-95% saturation; Bolton-Ritchie 2011b). Low oxygen levels result in a reduced number of invertebrate species and fewer individuals than some of the other sites (Bressington 2003). There is a high abundance of crustaceans, particularly the burrowing mudcrab *Macropthalmus hirtipes*, which contributes to approximately 30% of the invertebrate population (Bressington 2003). After the February 2011 earthquake, the new sediment mounds covered approximately 32% of this site, and were initially an average of 13 m in perimeter and 10 cm in height (Zeldis et al. 2011).

Plover Street (Figure 1.2a) is located on the eastern shore of the estuary, close to the mouth, and is representative of the eastern margin of the Estuary. It is located next to residential areas and is easily accessible by road, which makes it the site most likely to be disturbed by the public at low tide, for activities such as dog walking. BMA are usually visible as golden brown mats at the high tide level. Because this site is close to the mouth of the estuary, it tends to have lower nutrient levels (Knox & Kilner 1973), and higher salinity (26-33 ppt

during a tidal cycle) and dissolved oxygen levels (90-120% saturation) levels than the other two sites (Bolton-Ritchie 2011b). The seagrass *Zostera muelleri* forms dense beds in the mid shore level year round (Bressington 2003). *Ulva* sp. (5-50% cover) and *G. chilensis* (< 5% cover) are present at this site during summer, but disappear in the winter (Bressington 2003). Plover Street supports the highest number of invertebrate species and highest abundance of individuals compared to the other two sites and this is likely to be due to the presence of the seagrass beds, which are highly productive and contribute large amounts of detritus to the ecosystem (Bressington 2003). These beds also reduce the effects of waves and currents and provide a source of habitat heterogeneity (Bressington 2003). Gastropods, such as *Micrelenchus tenebrosus* and *Diloma* spp., are the most commonly found group of invertebrates (Bressington 2003). After the February 2011 earthquake, large volumes of raw sewage were continuously pumped into the high shore level at this site, until November 2011. Of the three sites studied, Plover Street had the highest percent cover of new sediment mounds (about 41%), and the lowest average mound height (about 6cm; Zeldis et al. 2011). New sediment mounds at this site were an average of 10 m in perimeter (Zeldis et al. 2011).

Humphreys Drive (Figure 2.1) is located on the southwestern side of the estuary, has a low current velocity (Knox & Kilner 1973), and is located closer to the oxidation ponds than the other two sites. The intertidal area is even in slope and covered by thick BMA films that are always visible on the old sediments and margins of the new sediments during low tide. The sediments are black and gelatinous, representing deposits of anoxic, fine sediments that were carried into this area from the old outfall site (Knox & Kilner 1973). Westward moving wind and currents caused the effluent from the old outfall site to become trapped at this site, and the sediments have accumulated large amounts of organic matter and nutrients (Knox & Kilner 1973). As a result of the wastewater influence, this site has the highest ammonium porewater concentration in the estuary (> 150 µg/ml; Knox and Kilner 1973), and low dissolved oxygen levels (79-135% saturation; Bolton-Ritchie 2011b). The low oxygen levels and high silt content make it difficult for invertebrates to survive here, and there is a reduced number of species and fewer individuals compared to the other two sites (Bressington 2003). Amphipods, such as *Melita awa*, are the most common invertebrate here (Bressington 2003). Westward moving currents previously resulted in the accumulation of dense *G. chilensis* (50-100% cover) and *Ulva* sp. (0-75%) beds at this site (Bressington 2003), but these macroalgae were no longer frequently visible during the time of this study. This is likely to be due to the tilting, burial and uplift of the estuary floor, and changes in tidal flows that occurred as a

result of the Christchurch earthquakes. The proximity of Humphreys Drive to river channels creates a low salinity range of 12-32 ppt during a tidal cycle (Bolton-Ritchie 2011b). New sediment mounds at Humphreys Drive covered approximately 35% of the surface, and were an average of 7 m in perimeter and 6 cm in height (Zeldis et al. 2011).

Figure 2.1: View of Humphreys Drive, showing brown mats of benthic microalgae lining the new sediment mounds that appeared during the February 2011 earthquake.

2.2.2 Monthly monitoring of benthic microalgal biomass in new and old sediments

In September 2011, three randomly selected new sediment mounds (> 2 m diameter) and adjacent areas of old sediment were selected at Humphreys Drive, Plover Street and the Heathcote River Mouth. All new sediment mounds were at least three sampling units away from each other, were located in the mid tidal level, and marked with bamboo sticks. The mid tidal level was chosen for sampling because it contained the greatest amount of new sediment mounds compared to other tidal levels and allowed for longer low tide sampling periods when compared to low tidal areas. During low tide, a contact core was taken from the top and margin of each new sediment mound and from the adjacent area of old sediment to assess BMA biomass (see Appendix 1 for contact coring method). Contact cores were taken again the following month, along with a sediment sample to analyse grain size and organic content (see Appendix 1 for processing of sediment samples). Samples were collected only on sunny

days, during the afternoon low tide period, after the sediment had been exposed to the air for at least two hours. The sampling procedure was repeated during the third week of each month, until March 2012, and maps of each area were drawn and marked to prevent sampling from occurring in the same place each month.

2.2.3 *In situ* effects of new sediment on benthic microalgal community composition

A 1 cm plastic corer (1.5 cm internal diameter) was fully inserted into the sediment beside each contact core taken at Humphreys Drive during monthly sampling in October 2011 and February 2012. The core was dug out and the sediment inside the core was pushed upward from the bottom so that the top 5 mm of sediment could be removed using a scalpel, and placed into a container with 15 ml of filtered estuary water (filtered through a 20 µm pleated polypropylene filter, a 5 µm polypropylene filter and finally a 0.9 µm ceramic filter). To remove large sediment particles, the sample was inverted ten times and allowed to settle for ninety seconds, similar to the method of Cane (1996), at which point 10 ml of the supernatant was carefully poured into a 10 ml measuring cylinder. Five millilitres were poured into a sterile plastic container and algae in the sample were stained and preserved with 3 drops of Lugol's Iodine solution (2.5%), and the other 5 ml was used to examine living microalgae.

To prepare a microscope slide for taxonomic identification, a sample was inverted ten times and a Pasteur pipette was used to take a drop from just below the sample surface. The drop was transferred onto a microscope slide and covered with a coverslip. Each sample was then viewed at 400x magnification using a Zeiss AxioImager.M1 compound microscope equipped with Differential Interference Contrast (DIC) illumination. Ten random fields of view, located by a random number generator, were used to identify microalgae to division. Only cells with visible plastids were identified. A total of 400 individuals were identified for each sample and, in some cases, this required the preparation of up to four microscope slides. Magnifications of up to 1000x were sometimes used to help identify individuals and photographs were taken using a Zeiss AxioCam HRc CCD camera with AxioVision Rel. 4.5 software (3900 x 3090 pixel resolution) to aid in identification. Algal units were counted, rather than cells, as per Cane (1991). A single unit differs from one species to another, but can consist of long filaments containing many cells, colonies of various sizes, and individual cells (Cane 1991).

The following references were useful in identifying individuals to division: Chrysophyta (Bold & Wynne 1986, Cane 1996, Hartley 1996, Hayward 1997), Chlorophyta (Bold & Wynne 1986, Ettl & Gartner 1995, van den Hoek et al. 1995, Cane 1996, Tomas & Hasle 1997), Euglenophyta (Pringsheim 1956, Leedale 1967, Cane 1996, Ciugulea & Triemer 2010), Cyanophyta (Humm & Wicks 1980, Komarek & Anagnostidis 1986, Anagnostidis & Komarek 1988, Hayward 1997).

2.2.4 Statistical analyses

2.2.4.1 Monthly monitoring of benthic microalgal biomass in new and old sediments

The effects of sediment type (old sediment, margin of new sediment mounds, and top of new sediment mounds), month (September/October 2011 – March 2012), and site (Heathcote River Mouth, Humphreys Drive, and Plover Street) on chlorophyll *a* (chl *a*) concentration (µg/g extract), percent organic content, percent coarse (>125-1000 µm diameter) and percent fine (0-125 µm diameter) sediment were tested using General Linear Models (GLMs). Chlorophyll *a* was analysed for the months from September 2011 to March 2012, whereas percent organic, coarse and fine sediment content were analysed for the months from October 2011 to March 2012. Sediment type and site were fixed factors and month was a random factor.

Separate one way Analyses of Variance (ANOVAs) were used to test for significant differences among sites and sediment types for five size fractions of sediments (500-1000, 250-500, 125-150, 63-125 and 0-63 µm), and correlation analyses were used to determine the relationship between each of the following: percent organics and percent fine sediments; percent organics and percent coarse sediment; and chlorophyll *a* and percent organics, fine and coarse sediment.

For all analyses in this experiment, homogeneity of variances was checked using Cochran's test and when necessary, data were transformed using $\log_{10}$- (x+1) for biomass and arcsin-square root for percentage data. Where this did not succeed in removing heterogeneity, the significant p-level was reduced from 0.05 to $p = 0.01$. Tukey's HSD post hoc tests were used when GLMs indicated significant effects. Data were analysed using STATISTICA 7.

2.2.4.2 In situ effects of new sediment on benthic microalgal community composition

The community composition within treatments (month and sediment type) was analysed using Permutation Analysis of Variance (PERMANOVA) and displayed graphically using Principal Components Analysis (PCO; Anderson 2001). This analysis was carried out on each month (October and February) separately, as well as on both months together. Data were transformed using fourth-root to down weight the importance of common divisions and increase the weight of those less abundant. Resemblance matrices were constructed using the Bray-Curtis measure of similarity, and all factors (month and sediment type) were fixed, with p-values obtained using 9999 permutations of the data.

Correlation analyses were performed between the following: number of Chrysophyta and percent organic content, coarse and fine sediment; number of Cyanophyta and percent organic content, coarse and fine sediment; number of Euglenophyta and percent organic content, coarse and fine sediment; and number of Chlorophyta and percent organic content, coarse and fine sediment.

All multivariate analyses were done using PRIMER 6 and PERMANOVA+, and correlations were analysed using STATISTICA 7.

2.3 RESULTS

2.3.1 Monthly monitoring of benthic microalgal biomass in new and old sediments

The range of chl *a* values obtained in this study were large, ranging from around 1 µg/g extract to 300 µg/g extract, and were highly variable (Figure 2.2). The interaction between sediment type and site determined the chl *a* concentration ($F_{4,126} = 15.898$, $p < 0.001$; Figure 2.2; Table 2.1), with all sediment types at Humphreys Drive generally containing higher concentrations of chl *a* when compared to all other sediment types at the other two sites (Tukey HSD; Figure 2.2). At Humphreys Drive, chl *a* was significantly higher in the margins, compared to the tops, of the new sediment mounds, and higher in old sediments compared to new sediment margins (Tukey HSD; Figure 2.2). Chlorophyll *a* concentrations did not differ significantly between months ($p > 0.01$), or on the tops of new sediment mounds between sites (Figure 2.2; Table 2.1).

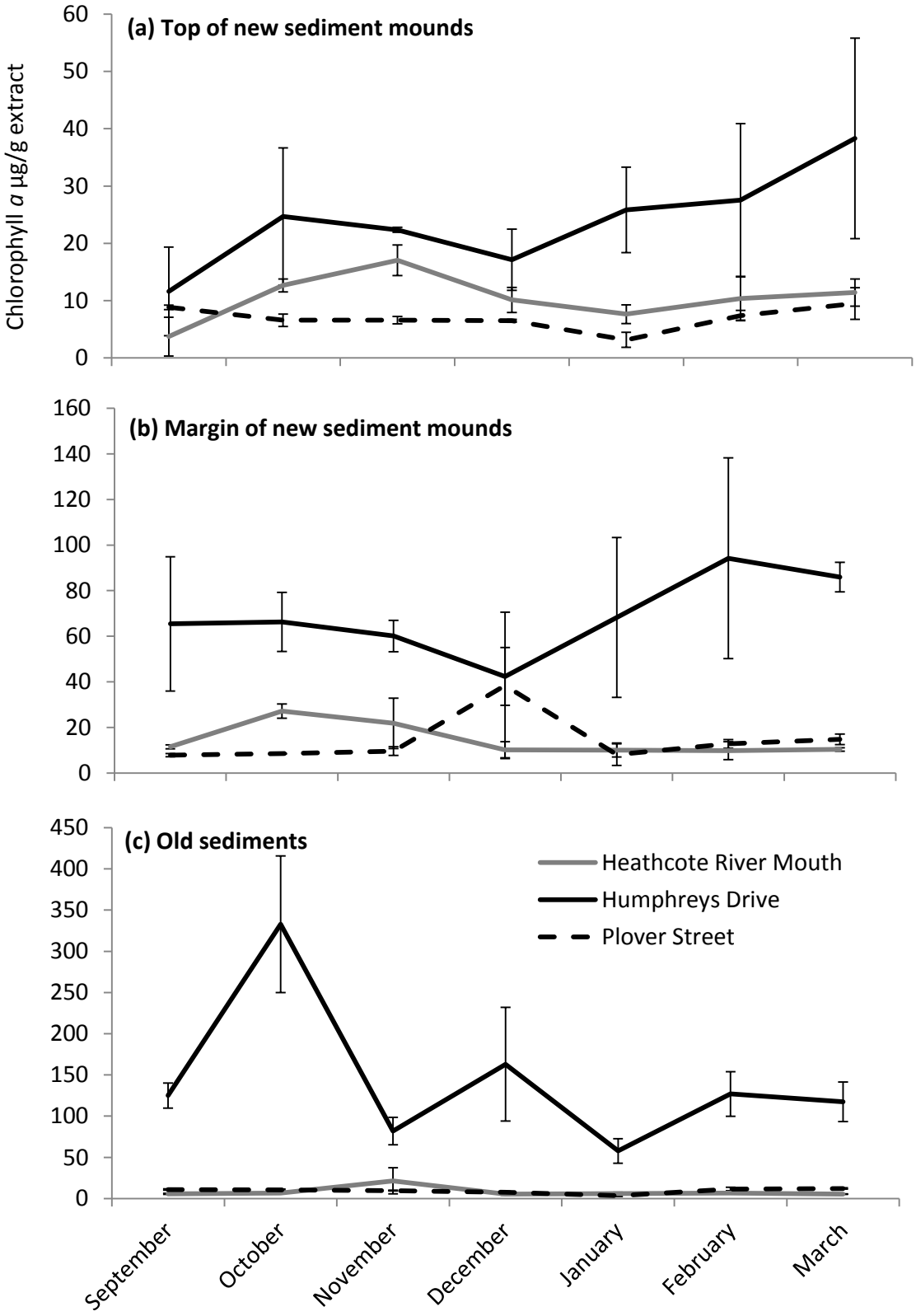

Figure 2.2: Mean (±SE) monthly (September 2011- March 2012) concentration of chlorophyll *a* (µg/g extract) at Heathcote River Mouth, Humphreys Drive, and Plover Street in the (a) top of new sediment mounds (b) margin of new sediment mounds and (c) old sediments. Note change in y axis scale between a, b and c.

Sediment in the 500-1000 µm size fraction was consistently the least common size class at all sites, whereas 125-250 µm sediment was the most common (Figure 2.3). Sediments larger than 1000 µm were not found in any sample and were not included in statistical analyses. Proportions of old sediment grain sizes were significantly different from site to site and in different sediment types (Table 2.2; Figure 2.3). Humphreys Drive showed the biggest difference in proportions of ambient grain sizes between sediment types, whereas Plover Street showed the least difference (Figure 2.3). In old sediments, Plover Street and the Heathcote River Mouth were dominated by the 125-250 µm size fraction, whereas sediments less than 125 µm were the most common at Humphreys Drive (Figure 2.3). Sediment profiles were similar on the tops of new sediments between all sites and had a higher proportion of coarser sediment size classes than areas with old sediment (Figure 2.3).

Table 2.1: General Linear Model results for the effect of different sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments), site (Heathcote River Mouth, Humphreys Drive, and Plover Street) and month (September 2011- March 2012) on the mean chlorophyll *a* concentration (µg/g extract).

Source of Variation	df	SS	MS	F	p
Month	6	1.56	0.26	2.592	0.122
Site	**2**	**22.39**	**11.19**	**103.301**	**0.000**
Sediment type	**2**	**3.02**	**1.51**	**25.306**	**0.000**
Month x Site	12	1.30	0.11	1.597	0.159
Month x Sed. Type	12	0.72	0.06	0.881	0.576
Site x Sed. Type	**4**	**4.32**	**1.08**	**15.898**	**0.000**
Month x Site x Sed. Type	24	1.63	0.07	1.160	0.291
Error	126	7.37	0.06		

The 0-63 µm and 63-125 µm size classes showed similar trends in most profiles and were proportionally higher than other size classes in old sediments (Figure 2.3). They were also both significantly higher in old sediments at Humphreys Drive and the Heathcote River Mouth when compared to tops and margins of new sediments (Tukey HSD; Table 2.2; Figure 2.3). Therefore, these size classes appeared to be good indicators of the pre-earthquake sediment composition and were pooled for statistical analyses and referred to as fine sediments (0-125 µm).

Table 2.2: Results of two-way ANOVAs examining effects of site (Humphreys Drive, Heathcote River Mouth, and Plover Street) and sediment type (top of new sediment mounds, margin of new sediment mounds, and old sediments) on each of five different grain-size fractions (0-63, 63-125, 125-250, 250-500, 500-1000 µm) of ambient sediments, from October 2011 to March 2012.

Grain size fraction	Site		Sediment type		Site x Sed. type	
(µm)	$F_{2, 153}$	p	$F_{2, 153}$	p	$F_{4, 153}$	p
500-1000 µm	8.61	< **0.001**	2.94	0.05	1.26	0.28
250-500 µm	26.23	< **0.001**	2.47	0.08	2.88	0.02
125-250 µm	47.65	< **0.001**	30.94	< **0.001**	6.58	< **0.001**
63-125 µm	17.04	< **0.001**	17.56	< **0.001**	6.59	< **0.001**
< 63 µm	3.27	0.04	31.4	< **0.001**	8.05	< **0.001**

When the remaining size classes (> 125-1000 µm) were pooled they were significantly more abundant in the tops of the new sediment mounds and did not differ significantly between sites (Tukey HSD; Figure 2.3), indicating that these sediments represented the liquefied portion of the substrate. Therefore, they were also pooled for statistical analysis and are referred to as the coarse sediments.

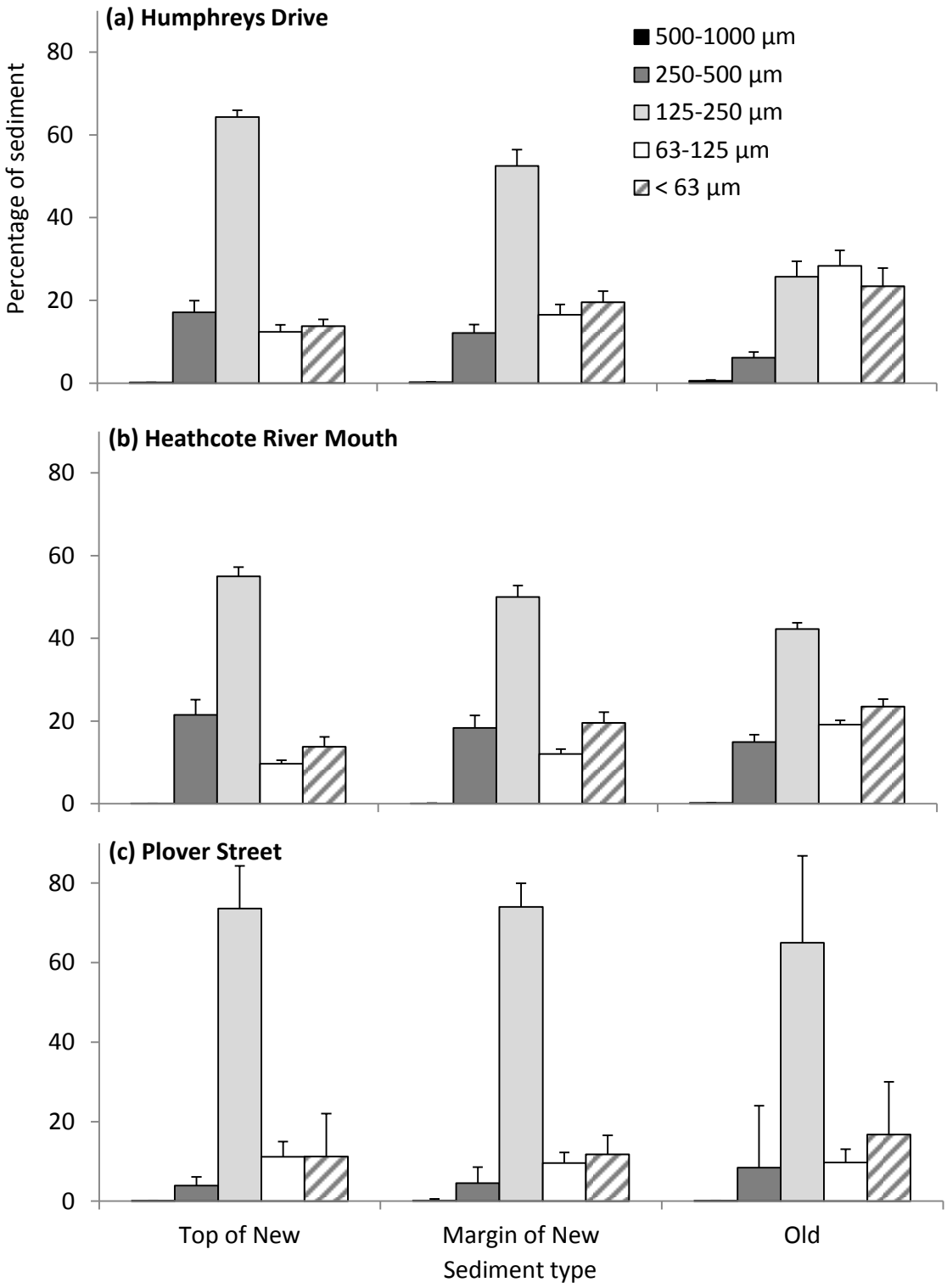

Figure 2.3: Mean (+SE) percentage of sediments of different grain sizes for different sites (Humphreys Drive, Heathcote River Mouth, and Plover Street) and sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments) from October 2011-March 2012.

The percentage of coarse sediment (> 125-1000 µm) was significantly affected by the interaction between site and sediment type ($F_{4, 108} = 23.69$, $p < 0.001$; Table 2.3a; Figure 2.4). There was a smaller percentage of coarse sediment in the old sediments at Humphreys Drive and the Heathcote River Mouth compared to all other sediment types at the other two sites (Tukey HSD). At Humphreys Drive, the proportion of coarse sediment was significantly higher in the tops, compared to the margins, of the new sediment mounds, and higher in old sediments compared to new sediment margins (Tukey HSD; Figure 2.4). Coarse sediment content at Plover Street did not differ between sediment types, but the coarse sediment content at the Heathcote River Mouth was lower in old sediment compared to the top of new sediment mounds (Tukey HSD; Figure 2.4). In the old sediments, Plover Street had the highest proportion of coarse sediments, followed by the Heathcote River Mouth and Humphreys Drive (Tukey HSD; Figure 2.4).

Table 2.3: General Linear Model results for the effect of different sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments), site (Heathcote River Mouth, Humphreys Drive and Plover Street) and month (October 2011- March 2012) on the mean percent (a) coarse (> 125-1000 µm) and (b) fine (0-125 µm) sediment.

(a) Mean percent coarse (>125-1000 µm) sediment

Source of Variation	df	SS	MS	F	p
Month	5	1397.7	279.5	5.60	0.043
Site	**2**	**2846.5**	**1423.2**	**27.04**	**0.000**
Sediment type	**2**	**6845.7**	**3422.8**	**88.77**	**0.000**
Month x Site	10	526.4	52.6	1.28	0.307
Month x Sed. type	10	385.6	38.6	0.93	0.524
Site x Sed. type	**4**	**3910.9**	**977.7**	**23.69**	**0.000**
Month x Site x Sed. type	20	825.3	41.3	0.48	0.968
Error	108	9211.3	85.3		

(b) Mean percent fine (0-125 µm) sediment

Source of Variation	df	SS	MS	F	p
Month	5	1785.4	357.1	3.87	0.064
Site	**2**	**1908.4**	**954.2**	**8.65**	**0.007**
Sediment type	**2**	**6653.2**	**3326.6**	**121.45**	**0.000**
Month x Site	10	1102.9	110.3	2.43	0.044
Month x Sed. type	10	273.9	27.4	0.60	0.793
Site x Sed. type	**4**	**3829.4**	**957.4**	**21.09**	**0.000**
Month x Site x Sed. type	20	908.0	45.4	0.69	0.828
Error	108	7099.6	65.7		

The interaction between site and sediment type also significantly affected the percentage of fine sediment (0-125 µm) ($F_{4, 108}$ = 21.09, p < 0.001; Table 2.3b; Figure 2.4), with similar patterns as with coarse sediment. However, wherever coarse sediment increased, fine sediment decreased. Month did not have an effect on either coarse or fine sediment (p > 0.01).

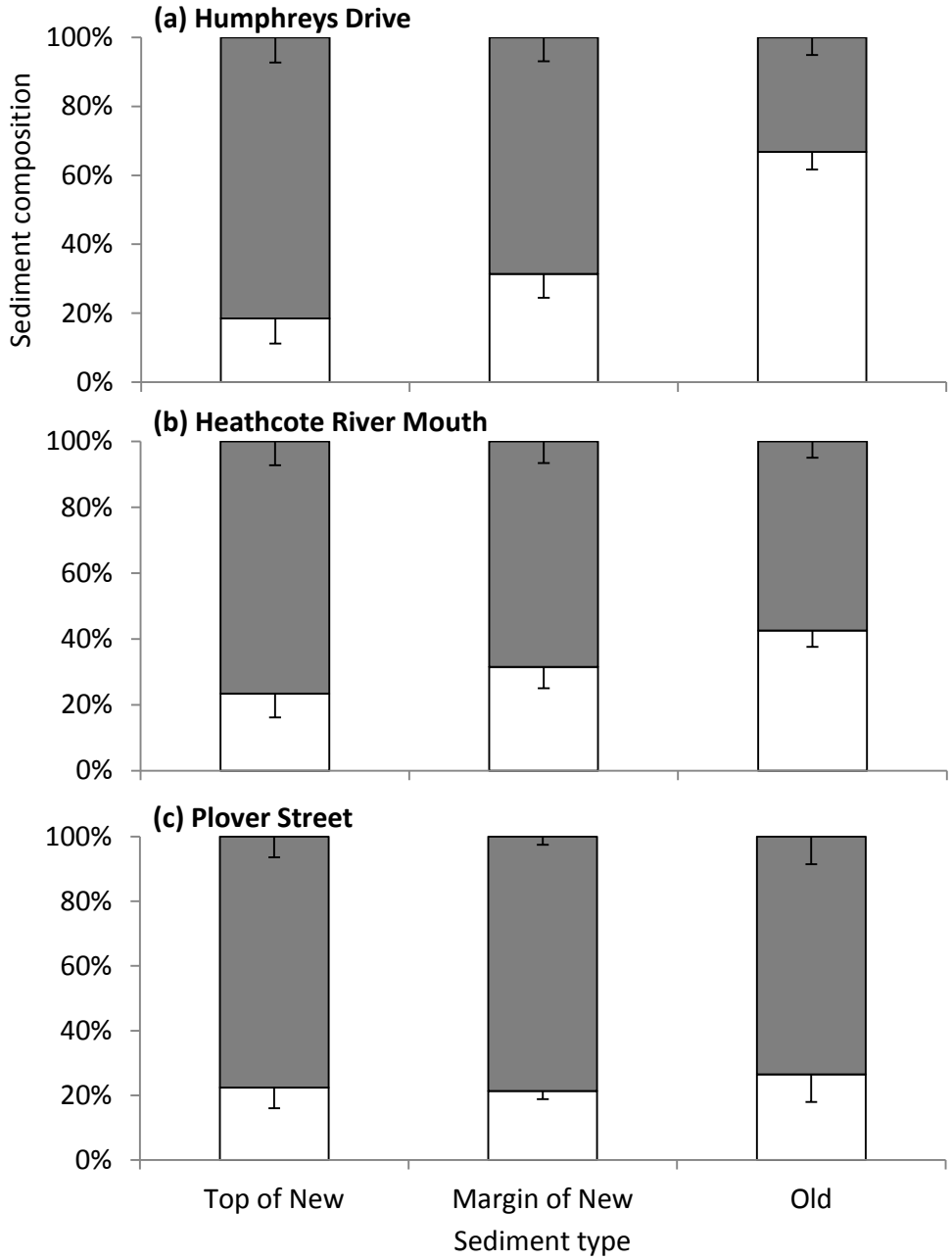

Figure 2.4: Proportion (-SE) of fine (0-125 µm) and coarse (>125-1000 µm) sediments compared between sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments) and sites (Heathcote River Mouth, Humphreys Drive and Plover Street). Data were averaged across months. Grey and white bars represent coarse and fine sediments, respectively.

The interaction between sediment type and site ($F_{4, 108} = 30.841$, $p < 0.001$; Figure 2.5; Table 2.4) significantly affected organic content, with higher organic content in the old sediments at Humphreys Drive compared to all other sediment types at the other two sites (Tukey HSD; Figure 2.5). The margins of the new sediment mounds at Humphreys Drive contained significantly more organic content than the tops of new sediment mounds at the same site (Tukey HSD; Figure 2.5). Organic content was not significantly different between sediment types at Plover Street or the Heathcote River Mouth, and did not differ in the tops of new sediment mounds between sites ($p > 0.01$). Month did not have a significant effect on organic content ($p > 0.01$).

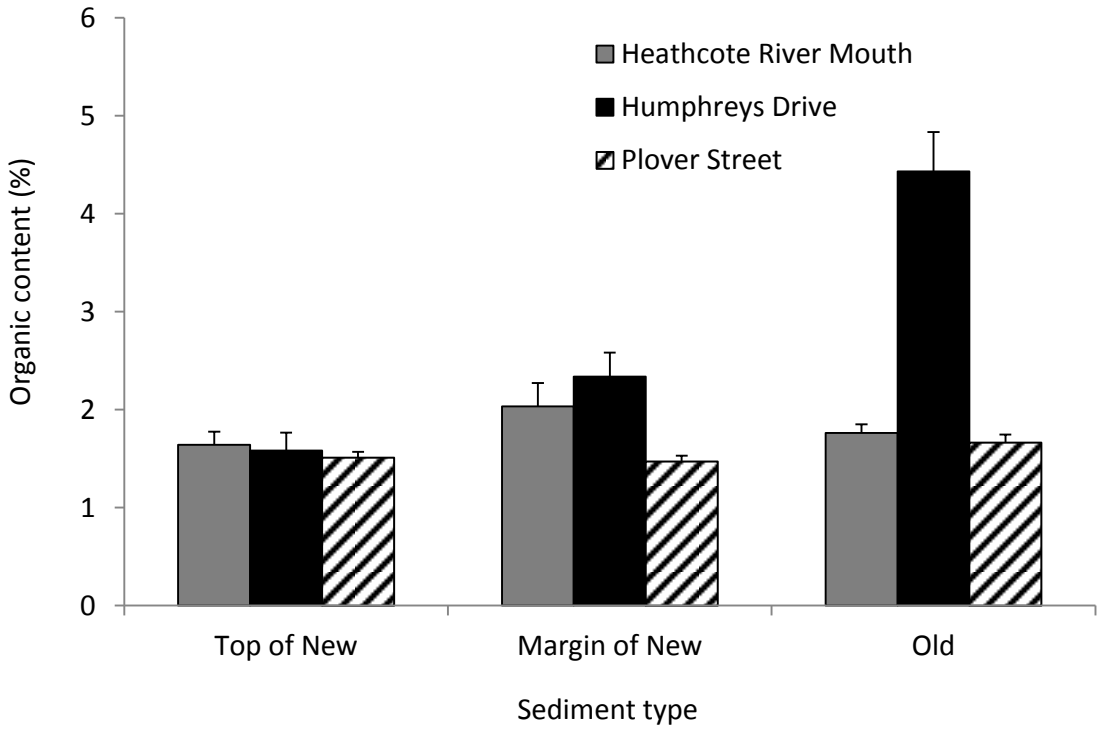

Figure 2.5: Mean (+SE) percent organic content in different sediment types (top of new sediment mounds, margin of new sediment mounds and old sediments) at three sites (Heathcote River Mouth, Humphreys Drive, and Plover Street). Data were averaged across months.

Table 2.4: General Linear Model results for the effect of different sediment types (top of new sediment mounds, margin of new sediment mounds and old sediments), site (Heathcote River Mouth, Humphreys Drive and Plover Street) and month (October 2011- March 2012) on the mean percent organic content in the sediment.

Source of Variation	df	SS	MS	F	p
Month	5	68.56	13.71	4.479	0.021
Site	**2**	**129.18**	**64.59**	**28.856**	**0.000**
Sediment type	**2**	**98.05**	**49.03**	**25.422**	**0.000**
Month x Site	10	22.38	2.24	2.024	0.086
Month x Sed. type	10	19.29	1.93	1.744	0.139
Site x Sed. type	**4**	**136.40**	**34.10**	**30.841**	**0.000**
Month x Site x Sed. type	20	22.11	1.11	0.626	0.885
Error	108	190.66	1.77		

Chlorophyll *a* was moderately, but significantly, correlated with fine sediment ($r_{162} = 0.43$, $p < 0.001$; Figure 2.6a), organic content ($r_{162} = 0.62$, $p < 0.001$; Figure 2.6b), and coarse sediment ($r_{162} = -0.52$, $p < 0.001$). Fine and coarse sediment had moderately strong positive ($r_{162} = 0.70$, $p < 0.01$; Figure 2.7a) and negative ($r_{162} = -0.69$, $p < 0.01$; Figure 2.7b) relationships, respectively, with percent organic content.

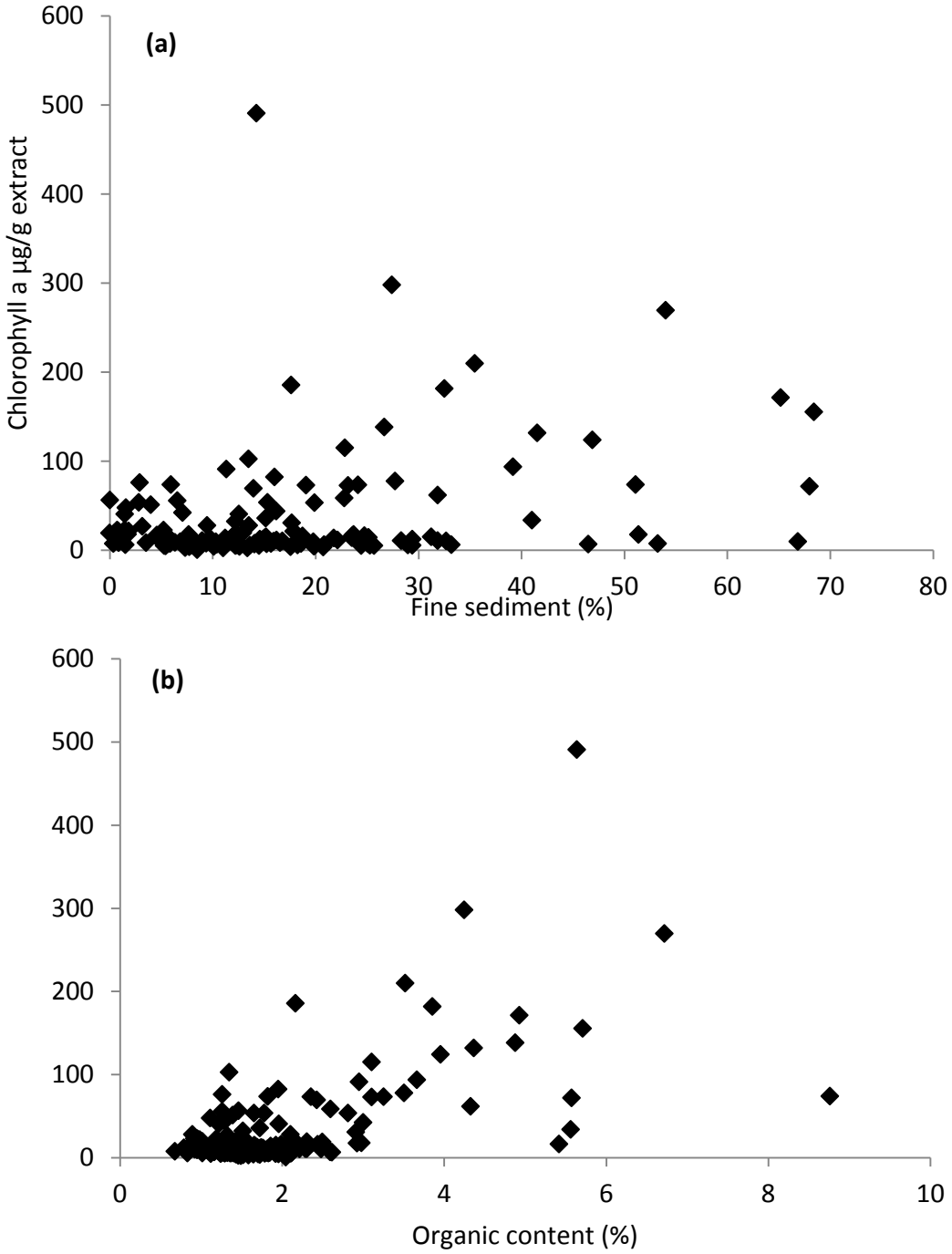

Figure 2.6: The relationship between chlorophyll *a* concentration (µg/g extract) and (a) percent fine (0-125 µm) sediment and (b) percent organic content for all sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments), months (October 2011- March 2012) and sites (Heathcote River Mouth, Humphreys Drive, and Plover Street), n = 162.

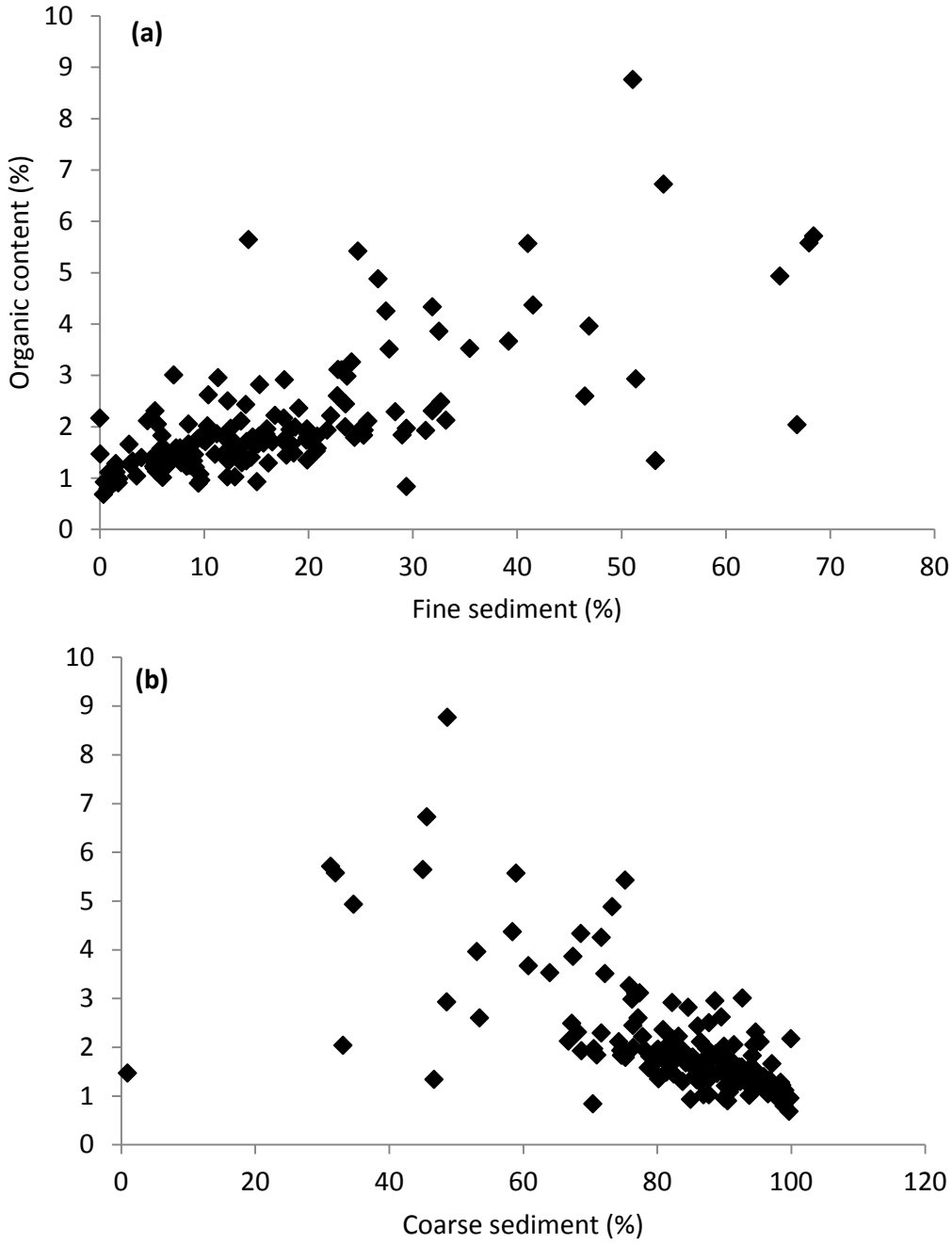

Figure 2.7: The relationship between percent organic content and (a) percent fine (0-125 µm) and (b) percent coarse (> 125-1000 µm) sediment for all sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments), months (October 2011- March 2012) and sites (Heathcote River Mouth, Humphreys Drive, and Plover Street), n = 162.

2.3.2 *In situ* effects of new sediments on changes in benthic microalgal community composition

A total of four microalgal divisions were recorded, with algae from each division present in every sample. The identified divisions were Cyanophyta (also known as cyanobacteria or blue-green algae), Chrysophyta (with diatoms as prominent members), Euglenophyta and Chlorophyta (green algae). Chrysophyta was the most common division (35-87% of samples), followed by Cyanophyta (11- 61%), Euglenophyta (1- 9%) and Chlorophyta (0.25-4%; Figure 2.8).

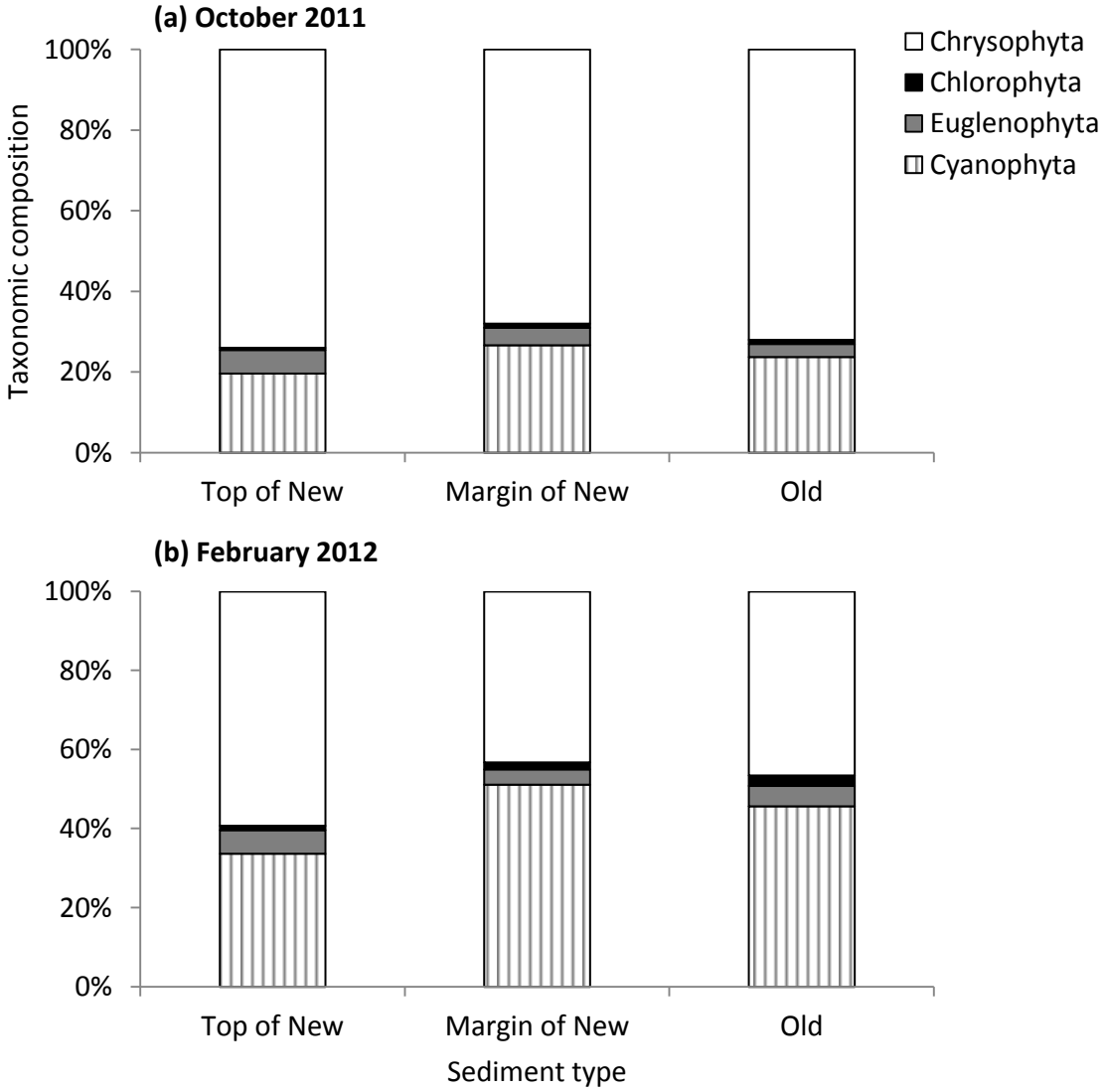

Figure 2.8: Proportion of benthic microalgal divisions (Chrysophyta, Chlorophyta, Euglenophyta, and Cyanophyta) at Humphreys Drive between sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments) in (a) October 2011 and (b) February 2012.

PERMANOVA showed differences in community composition between months (pseudo-$F_{1, 17}$ = 6.969, $p < 0.01$), with Principal Coordinates analysis illustrating that there were more Chrysophyta in October 2011 compared to February 2012 and more Cyanophyta in February 2012 compared to October 2011 (Figure 2.9). Sediment type did not have a significant effect on community composition ($p > 0.05$). Correlation analyses showed that there was a positive relationship between Chlorophyta and percent fine sediment ($r_{18} = 0.545$, $p = 0.019$), but no other significant relationships existed between divisions and physical variables. When PERMANOVA was used to analyse months separately, there were no significant differences in community composition between treatments ($p > 0.05$; Figure 2.10).

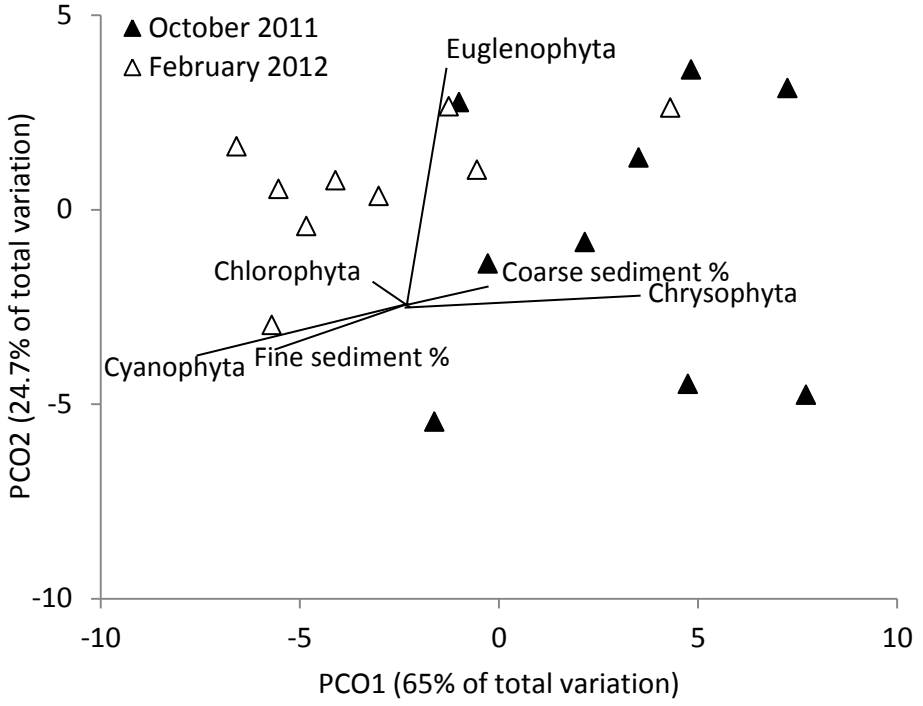

Figure 2.9: Principal coordinates biplot of taxonomic composition of estuary sediments in October 2011 and February 2012, across different sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments), with physical and biological predictor variables. Percentage of variation explained by individual axes is shown.

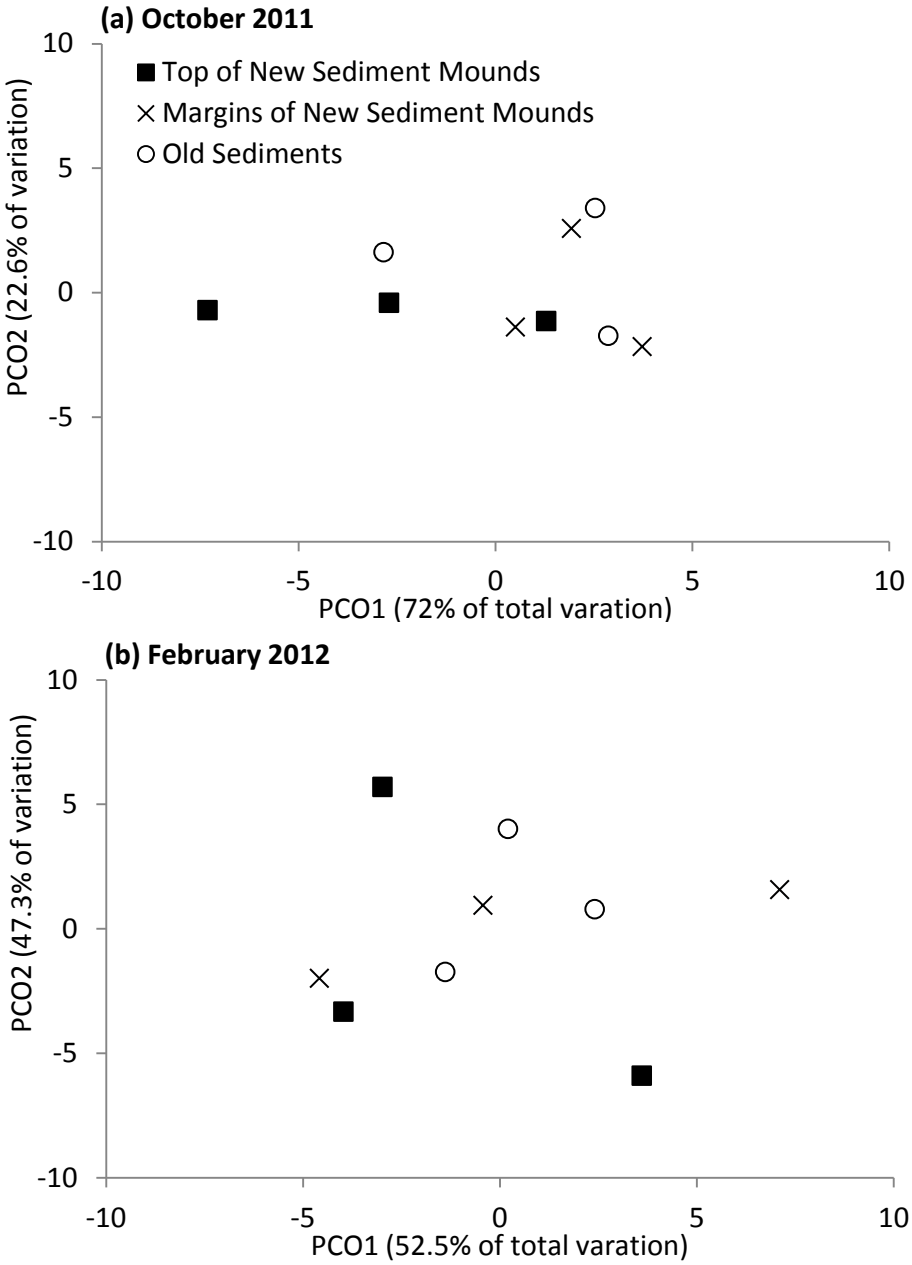

Figure 2.10: Principal coordinates biplot of taxonomic composition of estuary sediments across different sediment types (top of new sediment mounds, margin of new sediment mounds, and old sediments) in (a) October 2011 and (b) February 2012. Percentage of variation explained by individual axes is shown.

2.4 DISCUSSION

Sediment grain size profiles indicated that new sediments tended to have a greater proportion of coarse sediments when compared to old sediments, but that this was site-specific. While

the old sediments at Humphreys Drive and the Heathcote River Mouth contained significantly more fine sediments than the tops of the new sediment mounds at the same site, Plover Street did not show differences in grain size between sediment types. Before the earthquakes, there was an ambient transition from sand (> 63 µm diameter) at the mouth of the estuary to silt (< 63 µm diameter) at the head (Knox & Kilner 1973, Bolton-Ritchie 2011a), with the tidal reaches of the rivers also being particularly silty due to riverine influence (Owen 1992, Bolton-Ritchie 2011a). During this time, Plover Street was one of the sites in the estuary that contained the greatest percentage of sand compared to silt (80-100% sand), whereas Humphreys Drive and the Heathcote River Mouth contained larger proportions of silt (20-39% and 40-59% sand, respectively; Knox & Kilner 1973). This distribution was reflected in the old sediments in this study, where Plover Street contained a higher proportion of coarse sediments (around 73%) than the other two sites (around 32% at Humphreys Drive and 57% at the Heathcote River Mouth). In an estuarine environment, the distribution of sediment size depends on several factors such as water velocity, wind action and the hydrogeography of the area (Knox & Kilner 1973), with coarser particles reflecting deposition under high energy conditions and finer particles under low energy conditions (Flemming 2000). The coarse sediments found in the old sediments at Plover Street, which is close to the mouth of the estuary, were previously brought in by the tide from coastal beaches (Knox & Kilner 1973). The moderately well sorted nature of all sediment types at this site is due to strong tidal currents (Knox & Kilner 1973) and the instability and non-cohesive nature of the coarse sediments (Montserrat et al. 2008, Wardle 2009). The ambient coarse grain size and the high levels of mixing at this site probably account for the lack of difference in sediment size between old and new sediments. Differences in grain size were more easily seen at Humphreys Drive, where the old sediment had a higher proportion of fine sediment due to weak currents, shelter from wave action and historically high levels of pollution to aid in fine sediment flocculation and deposition (Knox & Kilner 1973). The low energy conditions at this site, indicated by the poorly sorted sediments in all sediment types and by the lack of significant difference in grain size proportions over time, would prevent mixing between new and old sediments. Similar mechanisms could account for the accumulation of fine sediments in the old sediment areas at the Heathcote River Mouth. However, most of the fine sediments at the Heathcote River Mouth were most likely supplied by the Heathcote River, which has been shown to carry at least 27 210 kg/day of sediment due to high sediment loading from the storm water drainage system that enters the river (Knox & Kilner 1973).

Organic content tended to follow the same trend as fine sediment, except at the Heathcote River Mouth where there were no differences in organic content between any of the three sediment types. Fine sediments are low in drainage and high in retained water due to their cohesive nature, whereas coarse sediments do not aggregate together as well, causing high drainage and lower retained porewater (Bressington 2003). Furthermore, organic content and fine particles are light and will settle out of the water column only if current turbulence is low (Morgans 1969). Therefore, it is not surprising that organic content shared a negative and positive relationship with coarse and fine sediment, respectively. Organic content in the tops of new sediment mounds was very similar between sites (1.5-1.6%) and was significantly higher only in the old sediments and margins of new sediment mounds at Humphreys Drive. This indicated that organic matter content in the new sediments did not depend on conditions in the local environment, and that although organic matter was able to accumulate in new sediments, a large proportion of it was potentially being transported back out of the sediments and into the water column due to the instability of the coarse sediments. Similarly, organic matter did not differ between sediment types at Plover Street, which is subjected to high energy currents (Knox & Kilner 1973). That sediment from the Heathcote River Mouth did not differ in organic content from sediment at Plover Street, or between sediment types within the same site is surprising given its high proportion of fine sediments in the old sediments and its proximity to the river. However, similar values to those in the old sediments at the Heathcote River Mouth have been noted before (Knox & Kilner 1973, Zeldis et al. 2011). It is possible that other environmental characteristics, besides grain size, are driving organic content distribution at this site. The higher percentage of organic content in the old sediments at Humphreys Drive compared to all other sediment types at the other two sites was likely due its close proximity to the old outfall site, which released organic rich effluent that tended to remain in the vicinity of the oxidation ponds without dispersing (Knox & Kilner 1973). Low current velocity and the previously large inputs of detritus from algal beds that used to accumulate at Humphreys Drive could also be responsible. In the Colne Estuary in the British Isles, Underwood et al. (1998) also found that sediment organic content was higher at sites in the vicinity of a wastewater outfall and that organic content decreased with increasing distance from these sites.

I also found that that BMA biomass and distribution were significantly affected by the appearance of the new sediments, and that this was correlated to the differences in grain size and organic content between new and old sediments. Greater BMA biomass tended to be

found in areas with fine sediment and/or high organic content. Other studies have also found relationships between BMA biomass and organic content (Light & Beardall 1998, Essink 2003) and grain size (Knox & Kilner 1973, Cartaxana et al. 2006, Brito et al. 2009, Jesus et al. 2009). The correlation of BMA biomass with organic matter was attributed to the microbial induced breakdown of organic matter into nutrients, which could then be used by BMA for growth (Light & Beardall 1998). The higher biomass of BMA at Humphreys Drive compared to the other sites is likely due to the higher amounts of organic breakdown, compared to other sites. Within this site, the higher biomass in the old sediments compared to the mound margins, and the mound margins compared to the mound tops, was also indicative of the BMA preference for greater amounts of fine, cohesive sediments (Wardle 2009). Fine sediments are more stable than coarse sediments and trap more water, which means that algae are more likely to be more protected from desiccation than in areas of coarse sediments (Brito et al. 2009). Coarse sediments are more porous than fine sediments, and therefore less stable, and increase the risk of BMA mats detaching from the sediment (Larson & Sundback 2012). Furthermore, BMA produce an extracellular polymeric substance (mucilage), which promotes the adherence of sediment particles by filling the interstitial spaces and decreases the occurrence of sediment resuspension (Montserrat et al. 2008). Mucilage could be less effective in binding sediments with larger interstitial sizes, thereby preventing BMA from being able to attach to the substrate and form stable colonies. The shape of the new sediment mounds could also be an important factor affecting the biomass of BMA. Anibal et al. (2007) found that in convex sediment shaped areas , which are similar in shape to the new sediment mounds, currents during high tide and desiccation during low tide were maximal and the higher hydrodynamic stress over these sections, compared to concave sections, made benthic life on these sediments difficult. This was explained by the low deposition of particulate organic matter and the export of detritus in convex areas, due to high current stress. It is important to note that causative interpretation of correlations between sediment size, organic content and the distribution of BMA is problematic because sediments with different grain sizes will also show interrelated differences in other physical and chemical variables, such as water column energy (Brotas et al. 1995, Grinham et al. 2011), salinity (Owen 1992), dissolved oxygen (Knox 1986, Bolam et al. 2000) nutrient fluxes and light penetration (Cahoon & Safi 2002). Therefore, the relationship between grain size and BMA biomass is complex and is influenced by a suite of complex interactions (Cartaxana et al. 2006).

Because of the observation that the shapes and sizes of some of the new sediment mounds were dynamic over time, and that several other small (< 0.5 m) new sediment mounds disappeared during the same time, it was surprising that analyses showed that grain size, organics and biomass did not change in the new sediments over the course of the study. It was also unexpected that these variables did not change in the old sediments over the period of the study, as it was expected that the new sediments would be resuspended by tidal action and distributed nearby in the old sediments. Additionally, ripples that were frequently present on the tops of new sediment mounds indicated that sediment transport was taking place (Montserrat et al. 2008). There are several reasons for the lack of significant change over time, but the most important ones are the patchiness of the BMA communities and the high variability in all of the measured variables each month. The effect of biotic and abiotic factors on temporal variation is difficult to test with statistical analyses, either because short term variability in estuaries blurs any temporal patterns (Garrigue 1998) or because other factors, such as animal activity (Davis & McIntire 1983), tidal coefficients (Jenness & Duineveld 1985) and grazing (Asmus 1982) enhance or disrupt peaks in benthic biomass . Furthermore, Colijn and Dejonge (1984) found that the large differences in biomass between only a few habitat types prevented statistical differentiation between these habitats. The lack of significant differences between months could also be due to the large size of the new sediment mounds chosen for this study (> 2 m in diameter), which appeared more stable in size and shape when compared to smaller ones at the same sites. Nonetheless, it was clear that at least the morphology of the sediment surface was changing over time because the new sediment mounds in this study eventually flattened slightly and appeared to expand outwards (Figure 2.11). Whether this is from coarse sediment being washed to sea or from local distribution over the old sediments is unclear. High variability in this study could indicate that sediment distribution was occurring locally, but unevenly, over the old sediments.

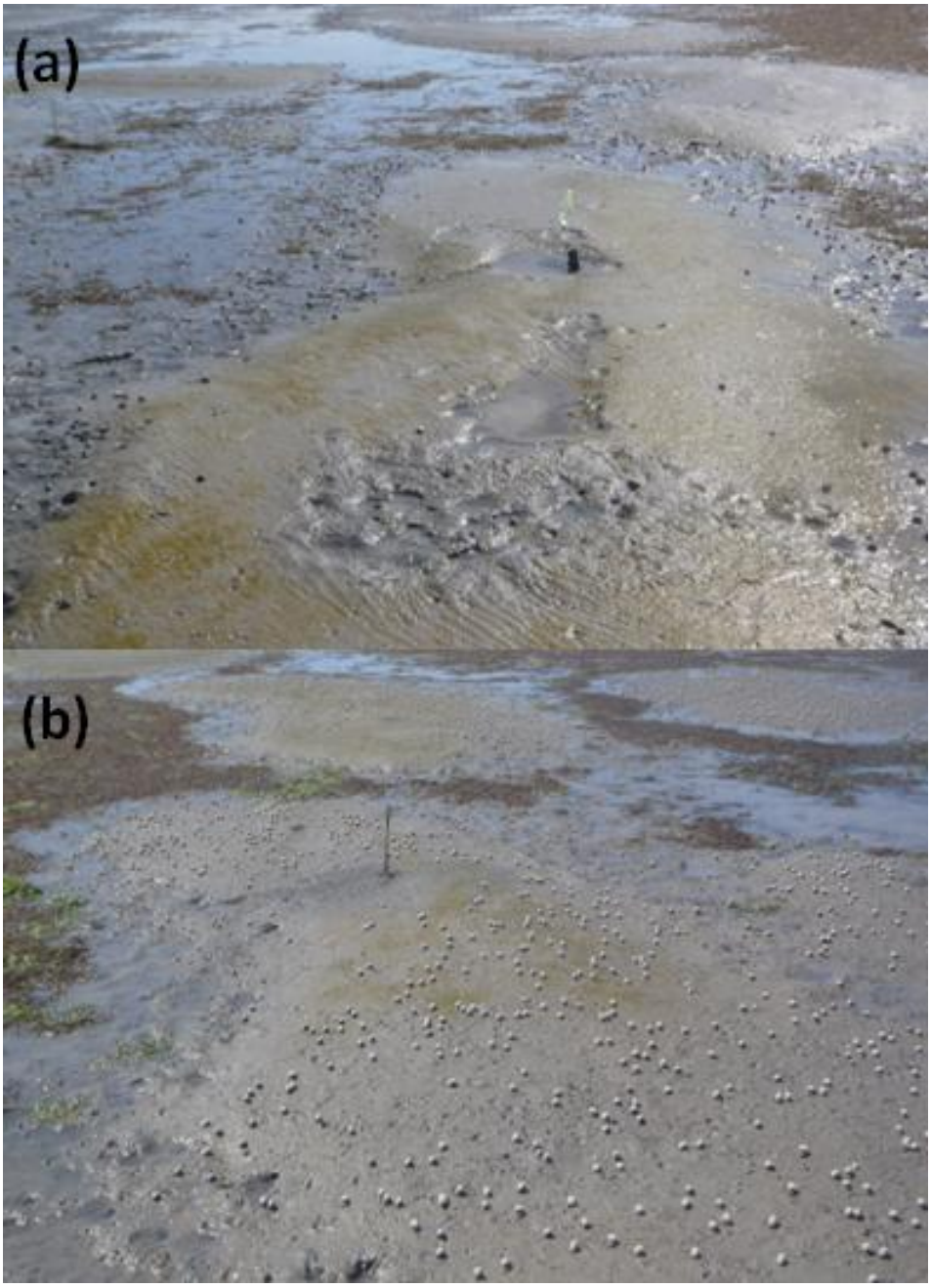

Figure 2.11: Photograph of a new sediment mound at the Heathcote River Mouth in (a) September 2011 and (b) February 2012. The mounds appeared to flatten and expand between these months.

Although biomass, grain size and organic content were not significantly different across months, BMA taxonomic composition was. Chrysophyta and Cyanophyta were more abundant in October and February, respectively. This could be indicative of a seasonal change because neither grain size nor organic content correlated with the abundance of these divisions. The correlation between fine sediment and Chlorophyta abundance is likely not indicative of a true ecological effect because chlorophyte abundances were always very low

(0.25-4% of the sample). The unimportance of sediment grain size on microalgal community composition was noted by Cartaxana et al. (2006), and increases in estuarine chrysophytes and cyanophytes in spring and summer, respectively, have also been seen (Pinckney et al. 1995). Cyanobacteria outcompete diatoms during high temperatures (> 20°C), while diatoms dominate during low temperature (10-15°C; Watermann et al. 1999). This could explain the higher proportion of cyanophytes during the summer, when daily temperatures in Christchurch frequently rose above 20°C, and the lower proportion of chrysophytes during spring when temperatures normally only reached 15°C. Cyanobacteria and diatoms are commonly the major constituents of intertidal microphytobenthos (Round 1981, Underwood & Kromkamp 1999, Cartaxana et al. 2006), and are particularly early colonisers (Janousek et al. 2007). Chlorophytes and euglenophytes have been shown to have a more variable and less widespread distribution in estuaries, and occur in localised patches that do not cover a large proportion of the sediment surface (Aneeshkumar & Sujatha 2012). In the AHE, these divisions were found at the old outfall site by Cane (1996), in much higher abundances than those found at the sites in this study.

Although a variety of other studies have found that grain size (Amspoker & McIntire 1978, Jesus et al. 2009) and organic content (Amspoker & McIntire 1978, Underwood 1997) determine community composition, it is hard to compare results due to the myriad techniques used to sample and analyse BMA. Furthermore, it has been shown that the effects of grain size on community assemblage depend on environmental conditions, such as temperature (Watermann et al. 1999), that are likely to change between estuaries. This study examined the community composition down to the division level, whereas most of the other cited studies investigated microalgal orders or classes, so it is possible that a change in taxonomic composition would have been detected in this study if finer taxonomic groups were investigated.

2.4.1 Summary

Patterns of BMA biomass and distribution after the earthquake-induced disturbances were context dependent. Generally, BMA biomass was significantly lower on the tops of new sediment mounds, compared to old sediments, and did not differ on mounds between sites, indicating that the new sediments are less favourable for BMA growth and recolonisation.

Lower biomass can lead to a compromised recovery from a eutrophic state, but this may not be the case in the AHE because new sediments tended to contain lower amounts of organic matter at the most eutrophic site. The emergence of less contaminated sediments may facilitate the recovery of the estuary by burying the old, more organically enriched surface, resulting in less of a need for high amounts of BMA nutrient cycling for recovery. Furthermore, the new sediments were similar to those at the least eutrophic site in all measured ways, indicating that they could bring the estuary towards a less eutrophied state. However, if the new sediments are being distributed over the old sediments, they could become as eutrophic as the old sediments and, due to grain size, could create a less favourable environment for BMA. In this case, recovery from eutrophication could be compromised. Ongoing research will determine which of these ensues.

CHAPTER 3

The effects of earthquake-driven sediment change and nutrient loading on benthic microalgae in a eutrophied estuary

3.1 INTRODUCTION

Coastal marine ecosystems worldwide are increasingly degraded as a result of excess nutrient loading from terrestrial sources, with signs of eutrophication being particularly evident in the estuaries of developed nations (Boesch 2002). For example, nearly one third of the estuaries along the American coast are highly eutrophied, with another third showing moderate symptoms of eutrophication (Bricker et al. 1999). The Chesapeake Bay Estuary in the eastern United States is an example of this, with eutrophication resulting in serious ecological problems, including harmful algal blooms and loss of aquatic vegetation. This has led to the implementation of several management strategies aimed at reducing nitrogen inputs into the estuary (Prasad et al. 2010). In southern New Zealand, the Avon-Heathcote Estuary (AHE) has experienced an increase in the supply of organic matter since the settlement of Christchurch, with the most important sources including sewage and catchment runoff through the Avon and Heathcote Rivers (Knox & Kilner 1973). The relatively enclosed waters of the estuary allow organic matter to settle into the sediment, where it is converted to ammonium-nitrogen via decomposition by microbes, such as bacteria (Graham & Wilcox 2000), and accumulates to high concentrations (> 200 µmol/L within the top 3 cm of sediment; Zeldis et al. 2011). Prior to the 2010 earthquake in Christchurch, the AHE was receiving more than 200 000 cubic metres of nitrogen rich water per day from the city's wastewater treatment plant and the two rivers (Bolton-Ritchie 2008). Consequently, ammonium concentration increased to the point where parts of the estuary were potentially toxic to marine life (Bolton-Ritchie 2008).

Of course, nitrogen is required by algae to manufacture amino acids and photosynthetic pigments and, therefore, is one of the most important nutrients required for growth and

primary productivity (McLusky 1971, Graham & Wilcox 2000). Nitrogen can be used by algae in the form of nitrate, nitrite or ammonium (Graham & Wilcox 2000), although algae preferentially use ammonium because it is a direct amino acid precursor and is a less energetically expensive source of nitrogen (Pomeroy 1959, Longphuirt et al. 2009). Consequently, with the addition of ammonium rich waters to the AHE over several decades, macroalgal blooms have repeatedly occurred. These eventually decay along the shoreline, causing the sediments in some areas to become anoxic and devoid of fauna (Bressington 2003). In the months following the wastewater diversion to an ocean outfall in March 2010, ammonium concentrations in the estuary fell to levels that were no longer toxic to estuarine life, but that changed in September 2010 when a series of earthquakes damaged sewer pipelines (Bolton-Ritchie 2011b). Large amounts of raw effluent and ammonium were released into the estuary, resulting in increased organic matter and nutrient supplies in the sediment (Zeldis et al. 2011).

Through the process of upward diffusion, advection, or microbial decomposition, sediment nutrients can be reintroduced into the overlying water column (Lyons et al. 1982) for months or years after nutrient loading changes, thereby delaying estuarine recovery (Hu et al. 2001). Recovery could also be altered by the emergence of new sediments if they carry different nutrient loads than old sediments. Heavily contaminated new sediments could present a eutrophication threat and further prolong the rehabilitation process, whereas less contaminated new sediments could accelerate recovery by burying pre-existing polluted sediments.

The extent of long term accumulation of nutrients in the sediments depends on factors that control nutrient cycling (Figueiredo da Silva et al. 2009). Benthic microalgae (BMA) influence the sediment-water nutrient flux through oxygenation by photosynthesis and uptake of nutrients for catabolic processes such as denitrification (Sundback et al. 2004). Denitrification reduces nutrients to nitrogen gas and releases them to the atmosphere, constituting an important pathway for removal of legacy nutrients from sediments. BMA, therefore, allow the system to resist eutrophication (Meyercordt & Meyer-Reil 1999). In the case of the AHE, the effects of BMA on recovery depend on whether they predominantly are using sediment (porewater) nutrients or nutrients in the overlying water column, and if these processes differ across new and old sediments. Ammonium is taken up by BMA cell walls from the immediate environment (Graham & Wilcox 2000), so the ability of BMA to use overlying water versus porewater will likely depend greatly on the direction of the diffusion

gradients between the overlying water column and the sediment that surrounds them. This is partially dependent on the concentrations of ammonium in the overlying water relative to the new and old sediments, and also on sediment porosity (Krom & Berner 1980). For example, diffusion rates could be higher in the new sediments, due to their coarser grain size and higher porosity (Zeldis et al. 2011).

Through recycling of nutrients in the sediments, BMA serve an important ecosystem function (Mann 2000), which can be measured by the fluxes of oxygen and concentrations of nutrients across the sediment-water interface (Lohrer et al. 2010). These variables are useful indicators of functioning because they are directly associated with habitat characteristics (Steffensen 1974, Lohrer et al. 2010) and help determine the system's overall photosynthetic rate and, hence, the ability to recovery from eutrophication. Before predictions about these processes can be made, however, the response of the BMA to the increased nutrient load requires experimental testing. In this chapter, I therefore address the following questions:

(1) Is there any nutrient based control over the distribution and abundance of BMA?
(2) Are BMA feeding more on porewater ammonium or ammonium from the overlying water column?
(3) Are BMA using nutrients from new and old sediments in the same way?

Although phosphorus and nitrogen are both important for growth and photosynthetic processes, phosphorous was not examined because it is not usually limiting in coastal waters and estuaries that experience a large influx of wastewater (Round 1981, Lohrer et al. 2010). Ammonium-nitrogen was investigated because it was in high enough concentrations in the estuary (> 5 µmol/L; Zeldis et al. 2011) that it was providing BMA with most of their required nitrogen (Admiraal et al. 1987).

To answer the above questions, the following hypotheses were tested:

(1) Microalgae would not be limited by nutrients because they would have access to high concentrations of ammonium in both sediment types. Because the AHE had been receiving waters with high organic and nutrient loads for the last 150 years (Bolton-Ritchie & Main 2005), sediment nutrient concentrations would be very high in the old sediments. The recent appearance of new sediments meant that ammonium levels would be lower in the new sediments, but as they were relatively porous and had been

present in the estuary during the large sewage inputs a year prior to these experiments, ammonium levels would still be much higher than that of the overlying water column.

(2) Porewater, compared to overlying water, would supply a greater amount of ammonium to microalgae. The diversion of wastewater to the ocean outfall had been reinstated in November 2011, resulting in lower inputs of nitrogen to the overlying water in this estuary. This, paired with high concentrations of legacy ammonium in the sediment, would create a diffusion gradient outwards, toward the top layer of sediment, where ammonium would be intercepted and fed upon by microalgae.

(3) Microalgae would use nutrients the same way in old and new sediments. As nutrients were not predicted to be limiting in either sediment type, it was expected that the biomass and, therefore, production values, would be similar and would be limited by other environmental factors, such as grazing.

3.2 METHODOLOGY

3.2.1 Laboratory based test of sediment type and ammonium concentration on benthic microalgal biomass

To test the interactive effects of sediment type and ammonium, an experiment was initiated in May 2012. Sediment cores were taken from a site with minimal infauna (Humphreys Drive) to minimise potential effects of small animals on chlorophyll *a* (chl *a*) concentrations during the experiment. Prior to the start of the experiment, oceanic water with a low ammonium concentration of around 2 µmol/L was collected 2 km outside of Lyttelton Harbour, brought back to the laboratory and filtered, first through a 1 µm mesh filter and then through a UV steriliser, for two days. Filtration of the water then continued throughout the duration of the experiment.

Sediment cores for the experiment were selected from 1 x 1 m plots within three patches with old sediment and three patches with new sediment. Plots were in the mid-tidal level and at least two meters from each other. Polyvinyl chloride (PVC) pipe with an internal diameter and length of 8 cm were used to take six sediment cores from within each plot. Cores from new sediments were taken from the tops of the sediment mounds. Pipes were inserted 4 cm deep into the sediment and a hand trowel was used to dig them from the ground. A PVC cap was placed on the bottom of each core and the cores were transported to a temperature controlled laboratory, set at 15°C (± 5°C). Cores were placed into their individual aquaria in

the tidal flow through system (see Appendix 1 for details on the tidal flow through system), their position previously determined by a random number generator. A potential artefact of this experiment was an uneven distribution of microalgae at the start of the experiment. To eliminate or diminish this possibility, the top 1 cm of sediment was scraped off of each core, mixed together and then reinfused as evenly as possibly by pouring aliquots of the mixture across all cores (see Appendix 1 for scraping and reinfusion methods).

The next day, three cores from each sediment type (new and old) were exposed to one of six nutrient levels. Each set of three replicate cores was fed from one bucket of source water, which contained f/2 media and one of the following ammonium-nitrogen concentrations: 2, 4, 8, 16, 32 and 64 µmol/L. The water for this experiment was collected from low nutrient waters (see above), and because I was only interested in the effect of nitrogen, I wanted to ensure that phosphorous was not in limiting supply in the source water. When nutrients are not limiting, the molar elemental ratio C: N: P in microalgae is 106: 16: 1 (Redfield 1934), so phosphorous was added in $1/16^{th}$ the proportion of ammonium in each treatment.

Prior to switching on the flow-through system, two 50 ml water samples were collected from each source bucket using syringes, and then filtered through 25 mm GF/F filters into acid cleaned containers. One filtered water sample was analysed for ammonium concentration, and the filter from the other sample was analysed for chl *a* as a proxy of microalgal biomass (see Appendix 1 for methods of processing chl *a* samples). The tidal and light cycles of the laboratory setup were then initiated, with each cycle mimicking that of the estuary for that time of year (10L: 14D and 6.25 h immersion: 6.25 h emersion).

The following day (about 24 hours after the initiation of the flow-through system), two more water samples were taken from each source water bucket and analysed for chl *a* and ammonium levels to determine the amount of change that had occurred in each. The water in each bucket was then replaced with new, filtered water and the ammonium solution was added again to each. Another two samples were taken from each bucket to determine initial amounts of chl *a* and ammonium and this cycle was repeated for five days, until the end of the experiment. On the final day, a contact core was taken from each sediment core to assess BMA biomass (see Appendix 1 for methods for processing chl *a* samples).

3.2.2 *In situ* test of sediment type and ammonium concentration on benthic microalgal biomass

On February 16, 2012 (here designated as week 0), two randomly selected plots of 80 x 80 cm, were marked within each of three patches with old sediment and three with new sediments at the Heathcote River Mouth. All plots were located in the mid tidal level and were marked at their two opposite corners using PVC piping. Of the two plots in each patch, one was marked as a control and one as an experimental plot. In each plot the percent cover of macroalgae and the abundance of *Amphibola crenata* were recorded. Three contact cores were taken from a random position in each plot for BMA biomass analysis (see Appendix 1 for methods processing chl *a* samples). To determine nutrient levels in the porewater, acrylic cylinders, 10 cm internal diameter x 30 cm deep, were used to take a sediment core from within each plot. The acrylic cylinders contained 3.1 mm diameter holes, which were positioned 1 cm apart, up the length of the cylinder. Prior to sampling, the holes had been covered with electrical tape to prevent the leakage of water and sediment. After the sediment cores were taken, electrical tape was cut away from the holes 1 cm above, immediately below, 1cm below and 2 cm below the sediment surface. Rhizone porewater samplers, product number 19.21.23F - http://rhizosphere.com/products, were attached to acid cleaned syringes and inserted into each hole. Seven millilitres of water was collected in each syringe and water was then transferred to 10 ml vacutainers and frozen until they could be analysed for ammonium concentration. Ammonium concentration was estimated with reference to standard curves as per the method of Koroleff (1983).

Prior to the incoming tide, the corners of each plot were marked with 1.5 m tall flags so plots could be located after tidal inundation. Animals and macroalgae were removed from the surface without greatly disturbing the sediment. Once the plots were covered by around 40 cm of water, two incubation chambers (one light and one dark) were placed in each plot to measure primary production via the oxygen exchange method (Figure 3.1a). The oxygen exchange method is the simplest technique for estimating benthic primary production and involves determining the oxygen flux across the sediment surface during illumination and dark incubation (Pomeroy 1959, Hunding 1973, Lohrer et al. 2010). Production and respiration both occur in the light chamber, while only respiration occurs in the dark chamber, and primary production is calculated as the difference in oxygen flux between these two incubations (Pomeroy 1959, Hunding 1973). Due to the limited time available during a single high tide period, separate light and dark chambers needed to be used to determine net

primary productivity, as per Lohrer et al. (2010). Chambers were plastic cylinders that were 16 cm tall and covered 0.0153 m^2 of the sediment surface, with space for 1 L of seawater above the sediment-water interface (Figure 3.1b). Two 5 mm internal diameter tubes (90 cm and 10 cm long) were connected to the chambers to enable sampling during tidal immersion. The longer tube was used for sampling and the smaller tube was to allow for water displacement as sampling was occurring. To cleanse all tubes prior to sampling, 50 ml of water was pulled through the chamber system using a syringe and then discarded before an initial sample of 50 ml was collected. Levels of dissolved oxygen were measured with a HACH HQ 30d probe. Collection times were recorded and final samples were taken from all chambers two hours later. To measure temperature and light availability, a HOBO data logger was placed inside one light chamber, one dark chamber, outside a light chamber and on the substrate on the edge of the estuary.

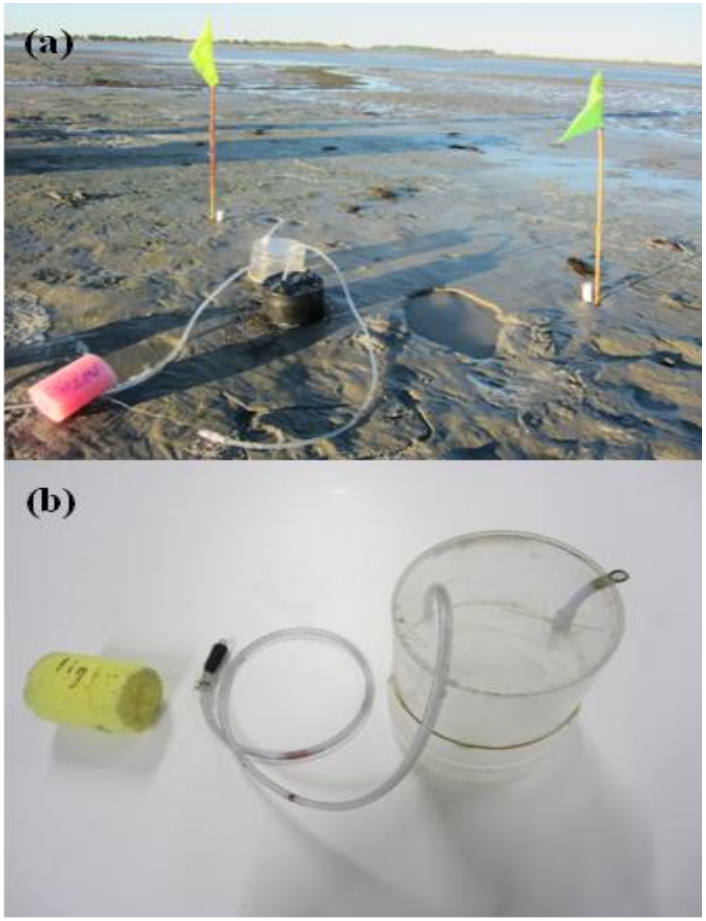

Figure 3.1: (a) A light and dark incubation chamber used to measure *in situ* primary production via the oxygen exchange method in experimental plots (80 x 80 cm) (b) A benthic chamber used to measure oxygen flux in the light.

The sediment underneath the chambers was collected during the following low tide period and put into a 500 µm mesh bag, which was used to sieve the sediment for infauna. Any individuals found were preserved in 70% ethanol. In the laboratory, samples were later placed in a Bogorov tray with ethanol and searched for animals using a LEICA DC 100 dissector microscope. The total invertebrate wet weight was recorded for each sample.

Because the experiment was set up to test varying levels of ammonium, the following procedure was used: immediately after the initial sampling procedure on week 0, experimental plots were sprayed with 2 L of site water, which had been concentrated to 64 µmol/L ammonium-nitrogen. This ammonium concentration was chosen not only to match the highest ammonium concentration from my laboratory nutrient experiment (Section 3.2.1), but because it was much higher (around 9 x) than the levels of ammonium in the overlying water at this site at the time (6 µmol/L, as determined by ammonium analysis one week prior to the experiment). *In situ* values of ammonium in coastal waters rarely rise above 30-40 µmol/L (L'Helguen et al. 1996), but the high levels used in this experiment provided an exceptionally high value to determine if BMA are nutrient limited in the AHE. Phosphorous was not added to this solution because it generally does not limit primary production in estuarine waters (Lohrer et al. 2010). Control plots were sprayed with 2 L of unaltered site water. All plots were sprayed every second day, for 21 days.

The entire sampling procedure was repeated once per week, for three weeks, and a random number generator was used prior to the experiment to determine where contact cores, sediment cores and production measurements would be taken from within the plots. Grids of each plot were drawn and marked to ensure that sampling did not occur in the same place in subsequent weeks.

3.2.3 Statistical Analyses

3.2.3.1 Laboratory analysis of sediment type and ammonium concentration on benthic microalgal biomass

The effect of ammonium concentration (2, 4, 8, 16, 32, and 64 µm/L) and sediment type (new and old) on sediment chl *a* concentration (µg/g extract) was tested using a General Linear Model (GLM), with both factors (nutrient level and sediment type) categorical and fixed.

3.2.3.2 In situ analysis of sediment type and ammonium concentration on benthic microalgal biomass

GLMs were used to determine if chl *a* was significantly different between control and fertilised plots at the start of the experiment, and between subsamples from each of the plots. Subsamples were treated as random factors, and if differences between them were non-significant ($p > 0.25$), they were pooled within treatments. Plots were regarded as random categorical factors when determining if chl *a* concentrations were significantly different between control and fertilised plots at the beginning of the experiment.

To test for significant effects of sediment type (old and new), fertilizing treatment (fertilised and control) and week (0,1,2 and 3) on chl *a* concentration and net primary production (NPP), two separate GLMs were used, with sediment type and fertilizing as fixed categorical factors, and week as a random categorical factor. Similar models were used to test for significant effects on sediment ammonium concentrations, the abundance of *A. crenata* on the surface of plots, the percent cover of macroalgae and the biomass of infauna. However, week was removed and depth (overlying, surface, 1 cm below, and 2 cm below) was added as a categorical fixed factor for the analysis on sediment ammonium concentrations, whereas only sediment type and fertilizing treatment were considered as factors when analysing the latter three.

Separate correlation analyses were used to determine if there were relationships between chl *a* and percent cover of visible macroalgae, abundance of *A. crenata* on the surface, infaunal biomass, and sediment nutrients at three depths (surface, 1 cm below, and 2 cm below). This type of analysis was also used to detect relationships between the following: NPP and chl *a*, infaunal biomass and macroalgal biomass; sediment nutrients at three depths (surface, 1 cm below, and 2 cm below) and abundance of *A. crenata* at the surface, macroalgal percent cover and infaunal biomass; macroalgal percent cover and abundance of *A. crenata* at the surface and infaunal biomass; and abundance of *A. crenata* at the surface and infaunal biomass. Where analyses indicated that relationships may be driven primarily by one sediment type (new or old), further correlation analyses were carried out comparing the variable of interest with each sediment type separately.

For all analyses in this chapter, homogeneity of variances was checked using Cochran's test and when necessary, data were transformed using $\log_{10}(x)$ or $\log_{10}(x+1)$ for all data except percent cover, which was transformed using arcsin-square root. Where this did not succeed in

removing heterogeneity, the significant p-level was reduced from 0.05 to p = 0.01. Tukey's HSD post hoc tests were used when GLM indicated significant effects. STATISTICA 7 was used to analyse data.

3.3 RESULTS

3.3.1 Laboratory analysis of sediment type and ammonium concentration on benthic microalgal biomass

The mean chl *a* concentration was significantly affected by the interaction between sediment type and ammonium concentration ($F_{5, 24} = 0.189$, p = 0.04; Figure 3.2). From an ammonium concentration of 16 µmol/L and higher, the mean amount of chl *a* tended to be greater in old sediments compared to new sediments. It was interesting that the mean amount of chl *a* in the old sediments generally increased from 4 µmol/L onwards and that chl *a* in the new sediments decreased abruptly after 8 µmol/L. However, it is noteworthy that the variance was high in almost all of the treatments and that a post hoc Tukey's HSD test comparing all group means from the sediment type and ammonium interaction were not significant for any pair of means. Furthermore, variability was largely heterogeneous between old sediments and new sediments, as well as between higher and lower ammonium concentrations.

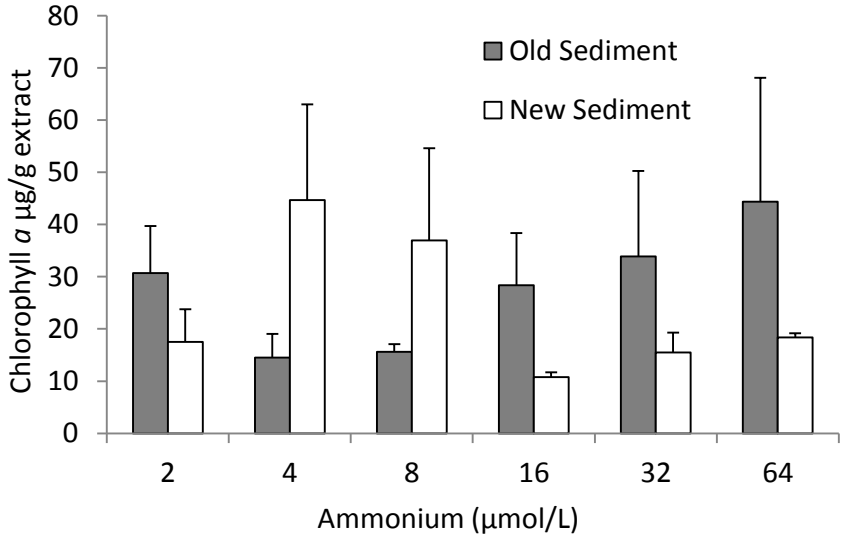

Figure 3.2: Mean (+SE) chlorophyll *a* concentration (µg/g extract) in cores (0.0045 m^2) across old and new sediments and ammonium concentrations (2, 4, 8, 16, 32, and 64 µmol/L) in the laboratory after 5 days.

Because replicates of the same treatment were fed from the same source bucket, there was no within treatment variance in source buckets. The following graphs indicate the changes in chl *a* (Figure 3.3) and ammonium concentration (Figure 3.4) in the source buckets over the course of the experiment.

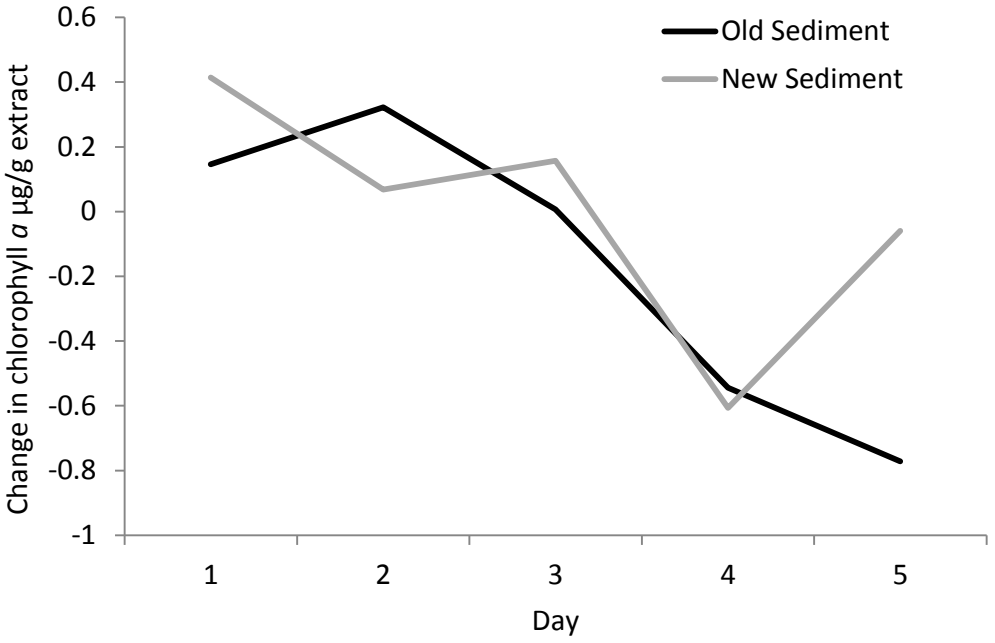

Figure 3.3: Change in chlorophyll *a* concentration (µg/L extract) between water replacements (every 24 hours) in source water buckets, for 5 days. Chlorophyll *a* concentrations were measured immediately after water was replaced, and 24 hours later, before the next water replacement. Data are averaged across all buckets containing different ammonium-nitrogen concentrations (2, 4, 8, 16, 32, and 64 µmol/L). Each bucket supplied cores with either new or old sediments.

Based on graphical analysis, it appeared that chl *a* levels in the source water buckets did not dramatically change (< 1 µg/L; note small values on y-axis of Figure 3.3) each day between water replacements and were similar for buckets connected to both new and old sediments. Trends were consistent across nutrient levels, so data were averaged across nutrient levels for each sediment type and presented as one graph (Figure 3.3). Therefore, it appeared that there was little leakage of BMA from the sediment cores in the experiment.

Changes in the ammonium concentrations in the source water buckets increased greatly between water replacements over the first two days in buckets connected to both sediment

types, until ammonium levels began to decrease on day 3 in buckets connected to new sediments, compared to day 4 in buckets connected to old sediments (Figure 3.4). Each bucket showed similar changes in ammonium each day, so data was averaged across nutrient levels for each sediment type and presented as one graph (Figure 3.4). It therefore appeared that ammonium was diffusing out of the sediment cores and into the overlying water column until at least day 3.

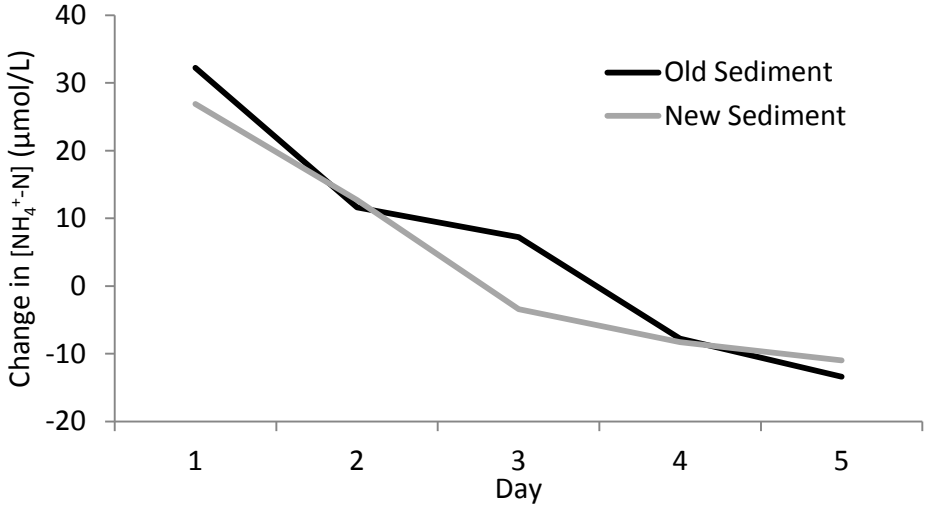

Figure 3.4: Change in ammonium-nitrogen concentration (µmol/L) between water replacements (every 24 hours) in source water buckets, for 5 days. Ammonium levels were measured immediately after water was replaced, and 24 hours later, before the next water replacement. Data is averaged across all buckets containing different ammonium concentrations (2, 4, 8, 16, 32, and 64 µmol/L). Each buckets supplied cores with either new or old sediments.

3.3.2 In situ analysis of sediment type and ammonium concentration on benthic microalgal biomass

Chlorophyll *a* concentration in the sediment was not significantly different between fertilised and control plots at the start of the experiment ($p > 0.05$), or between subsamples within each plot ($p > 0.25$), so subsamples were pooled within treatments.

Sediment type had a significant effect on chl *a* concentration ($F_{1, 128} = 49.36$, $p < 0.01$; Figure 3.5; Table 3.1a), with chl *a* being nearly more than twice as high in new, compared to old, sediments (Tukey HSD; Figure 3.5). Sediment type accounted for 21% of the variation in chl *a* concentration (Table 3.1a). Fertilizing treatment and 'week' failed to have a significant

effect on chl *a* concentration in the sediments ($p > 0.05$) throughout the experiment, but around 25% of the variation in chl *a* was accounted for by changes over time. By the third week, in particular, new and old sediments showed an upsurge in chl *a* in both treatments.

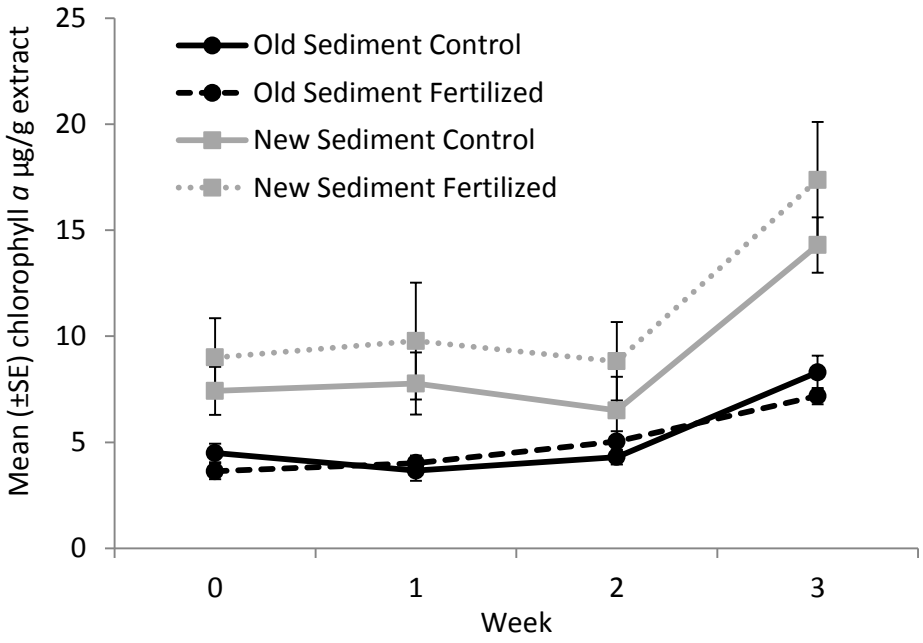

Figure 3.5: Mean (±SE) chlorophyll *a* concentration (µg/g extract) over 3 weeks in *in situ* plots, across different sediment types (new an old) and fertilizing treatments (fertilised and control).

There were significant, but generally weak to moderate, correlations between chl *a* concentration and infaunal biomass ($r_{48} = -0.50$, $p < 0.001$; Figure 3.6a), macroalgal percent cover ($r_{48} = -0.29$, $p = 0.04$; Figure 3.6b), abundance of *A. crenata* ($r_{48} = -0.56$, $p = 0.01$; Figure 3.6c), and sediment ammonium-nitrogen at the surface level ($r_{48} = -0.28$, $p = 0.049$; Figure 3.6d). When these correlations were analysed separately between old and new sediments, there were no longer significant relationships between chl *a* and infaunal biomass, macroalgal percent cover and sediment ammonium-nitrogen ($p > 0.05$ for all), and abundance of *A. crenata* remained significantly correlated with chl *a* in new sediments only ($r_{24} = -0.57$, $p = 0.003$).

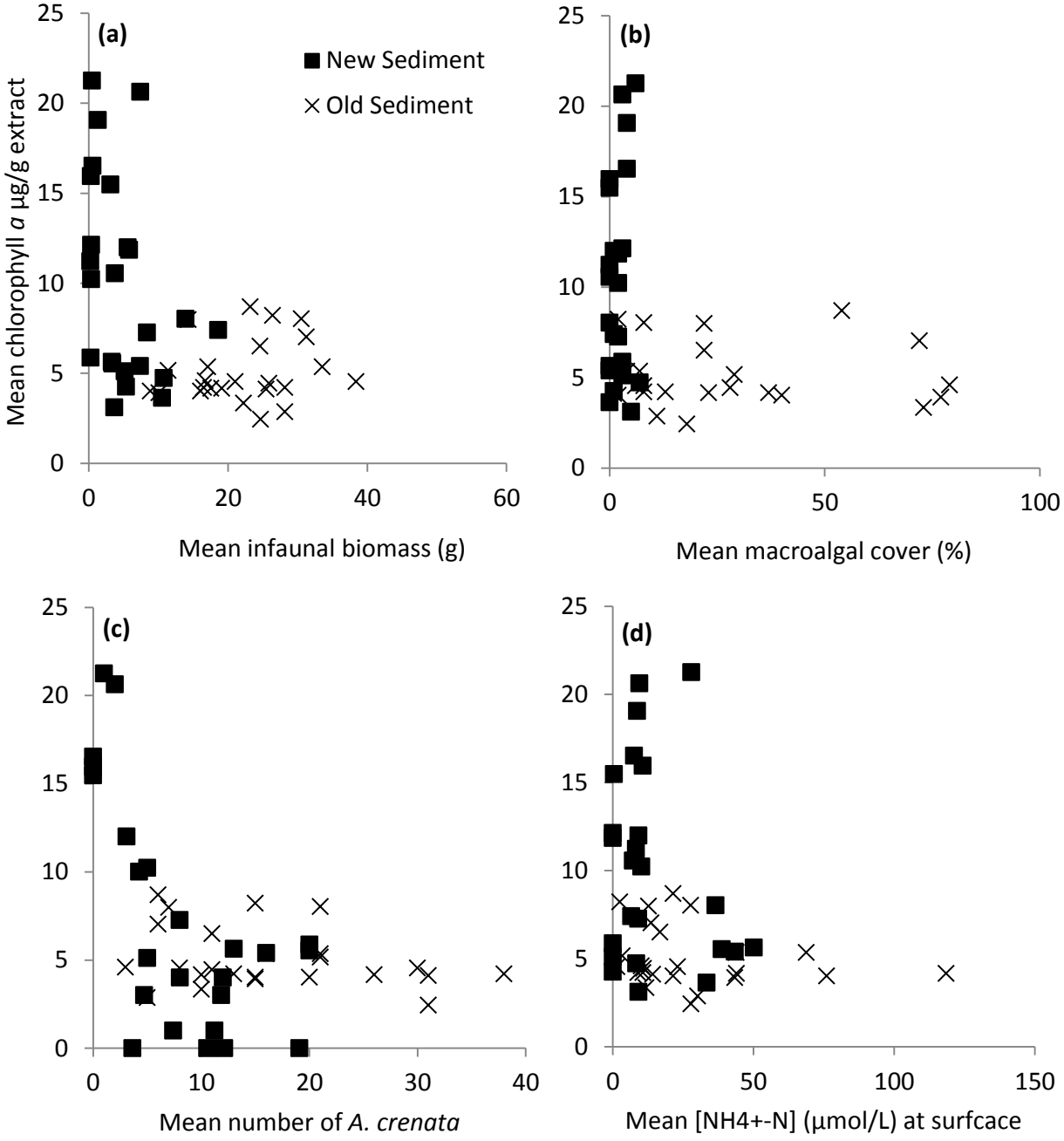

Figure 3.6: Mean chlorophyll *a* concentration (µg/g extract) per plot and mean (a) infaunal biomass (g) (b) macroalgal cover (%) (c) number of *Amphibola crenata* and (d) concentration of ammonium at the surface of the sediment, n = 48 for all.

The interaction between week and sediment type had a significant effect on net primary production ($F_{3, 32}$ = 10.397, p = 0.04; Figure 3.7; Table 3.1b), with differences being driven by increases in net production during week 3 in the old sediments (Tukey HSD; Figure 3.7). Initial correlations between NPP and infaunal biomass, and chl *a* were not significant (p > 0.05 for all). When correlations were rerun with new and old sediments separately, NPP in old sediments became significantly correlated with chl *a* (r_{24} = 0.76, p < 0.01; Figure 3.8).

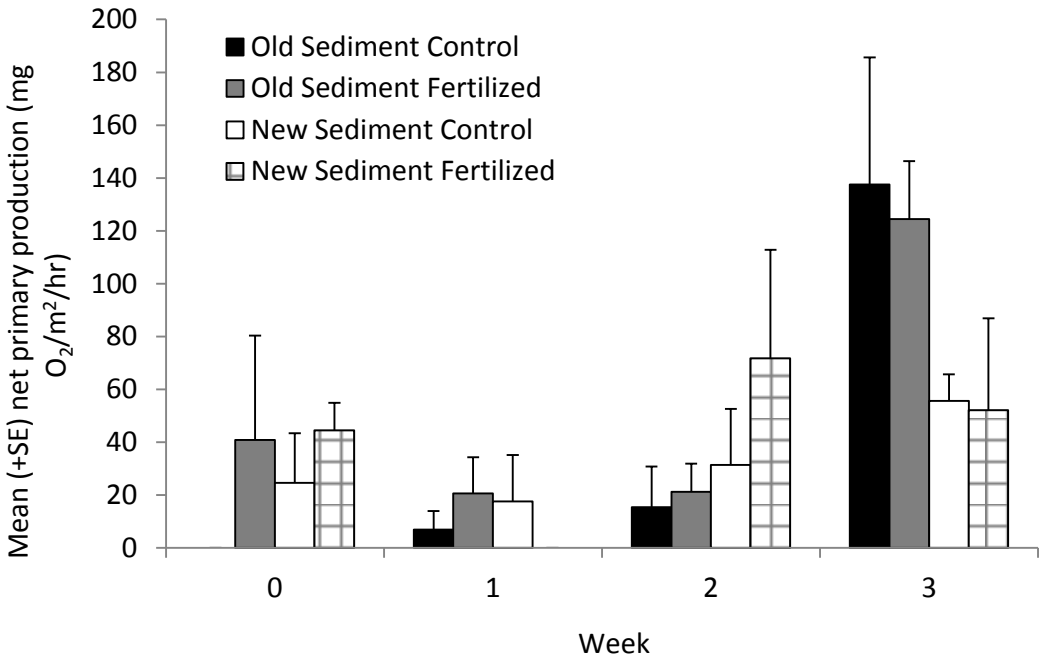

Figure 3.7: Mean (+SE) net primary production (mg $O_2/m^2/h$) over 3 weeks in *in situ* plots (0.64 m^2), across different sediment types (new an old) and fertilizing treatments (fertilised and control).

Table 3.1: General Linear Model results for the effect of different sediment types (new and old), fertilizing treatments (fertilised and control) and week (0, 1, 2, and 3) on the (a) mean concentration of chlorophyll *a* (µg/g extract) and (b) net primary production (mg $O_2/m^2/h$) in 0.64 m^2 *in situ* plots.

(a) Mean concentration of chlorophyll *a* (µg/g extract)

Source of Variation	df	SS	MS	F	p
Week	3	2.57	0.86	13.620	0.014
Sediment type	**1**	**2.22**	**2.22**	**49.369**	**0.006**
Fertilizing treatment	1	0.06	0.06	1.936	0.258
Week x Sed. type	3	0.13	0.04	4.230	0.133
Week x Fert. treatment	3	0.09	0.03	2.685	0.219
Sed. type x Fert. treatment	1	0.08	0.08	7.282	0.074
Week x Sed. type x Fert. treat.	3	0.03	0.01	0.274	0.844
Error	128	4.96	0.04		

(b) Net primary production (mg $O_2/m^2/h$)

Source of Variation	df	SS	MS	F	p
Week	3	44941.0	14980.3	2.035	0.28
Sediment type	1	903.9	903.9	0.130	0.74
Fertilizing treatment	1	1404.3	1404.3	1.326	0.33
Week x Sed. type	**3**	**20913.7**	**6971.2**	**10.397**	**0.04**
Week x Fert. treatment	3	3178.4	1059.5	1.580	0.36
Sed. type x Fert. treatment	1	13.0	13.0	0.019	0.90
Week x Sed. type x Fert. treat.	3	2011.5	670.5	0.390	0.76
Error	32	54959.4	1717.5		

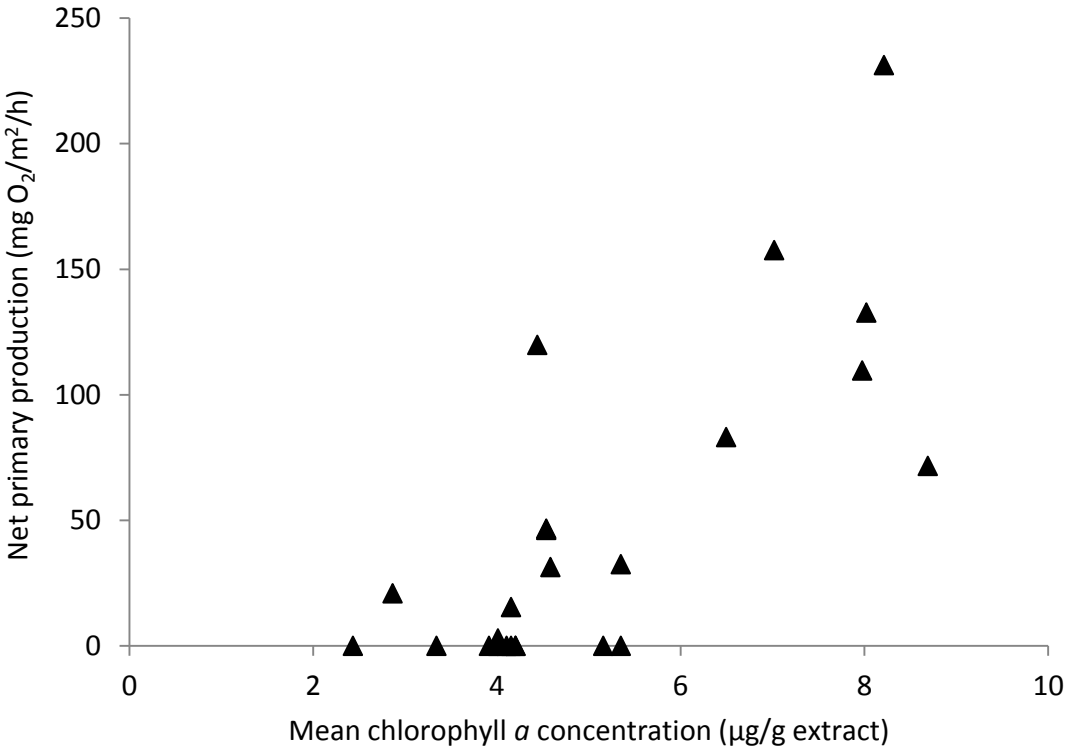

Figure 3.8: Mean chlorophyll *a* concentration (µg/g extract) and mean net primary production (mg $O_2/m^2/h$) in old sediments, n = 48.

Ammonium concentration increased with sediment depth ($F_{3, 176} = 62.37$, $p < 0.001$; Figure 3.9; Table 3.2), but was not significantly different between the overlying water and the sediment surface (Tukey HSD; Figure 3.9). The overlying water and surface sediment had relatively low concentrations of 20 µmol/L or less, but these increased 5 to 8 fold in 1 cm and 2 cm deep sediments. Generally, old sediments had a greater mean concentration of ammonium then new sediments ($F_{1, 176} = 35.05$, $p < 0.001$; Figure 3.9; Table 3.2).

Ammonium-nitrogen concentration was not correlated with infaunal biomass, abundance of *A.crenata* or percent cover of macroalgae at any depth ($P < 0.05$).

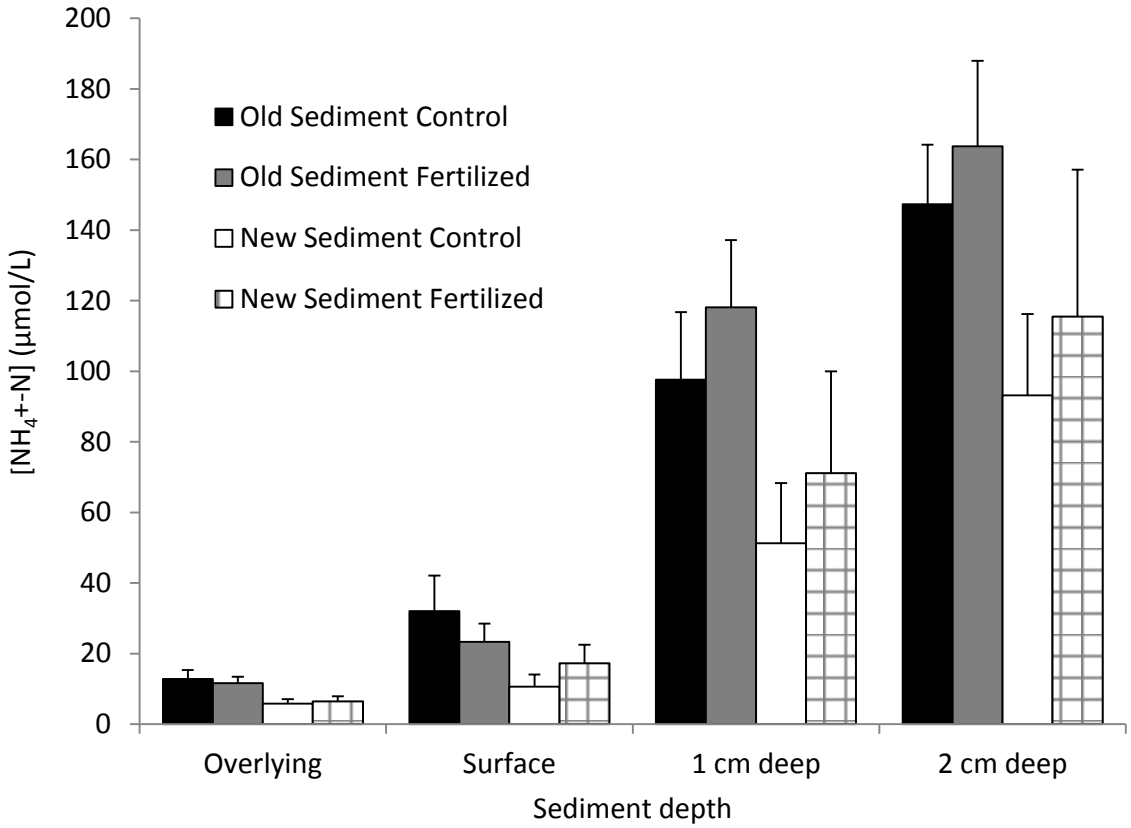

Figure 3.9: Mean (+SE) ammonium-nitrogen concentration (µmol/L) in plots (0.64 m^2) over 3 weeks, across different sediment types (new an old), fertilizing treatments (fertilised and control) and depths (overlying, surface, 1 cm deep, and 2 cm deep).

GLMs indicated that percent cover of macroalgae ($F_{1, 44} = 34.65$, $p < 0.001$), infaunal biomass ($F_{1, 44} = 73.71$, $p < 0.001$), and abundance of *A. crenata* on the sediment surface ($F_{1, 44} = 29.73$, $p < 0.001$) were significantly increased on old sediments compared to new sediments and that fertilizing treatment did not have significant effects ($p > 0.05$).

Significant correlations were found between infaunal biomass ($r_{48} = 0.47$, $p = 0.001$) and percent cover of macroalgae ($r_{4\ 8} = 0.32$, $p = 0.023$), and infaunal biomass and abundance of *A. crenata* ($r_{48} = 0.47$, $p = 0.001$).

Table 3.2: General Linear Model results for the effect of different sediment types (new and old), fertilizing treatments (fertilised and control), and depth (overlying, surface, 1cm below, and 2cm below) on the mean concentration of ammonium-nitrogen (μmol/L) in 0.64 m^2 *in situ* plots over 3 weeks.

Source of Variation	df	SS	MS	F	p
Sediment type	**1**	**7.00**	**7.00**	**35.06**	**0.000**
Fertilizing treatment	1	0.20	0.20	1.01	0.317
Depth	**3**	**37.35**	**12.45**	**62.37**	**0.000**
Sed. type x Fert. treatment	1	0.02	0.02	0.11	0.735
Sed. type x Depth	3	0.04	0.01	0.07	0.973
Fert. treat. x Depth	3	0.07	0.02	0.12	0.948
Sed. type x Fert. treat. x Depth	3	0.10	0.03	0.17	0.916
Error	176	35.14	0.20		

3.4 DISCUSSION

The chl *a*, porewater ammonium concentration, and production values seen in both *in situ* and lab experiments, although high in some cases, were comparable to those previously reported for estuaries in New Zealand and elsewhere (Rizzo 1990, Barranguet et al. 1996, Longphuirt et al. 2009, Lohrer et al. 2010). The laboratory experiment showed that overall the addition of ammonium to the water column did not have a significant ecological effect on the biomass of BMA, but also that BMA on old and new sediments may be using water column ammonium differently. The field based experiments also showed differences between old and new sediments, but no significant effect of elevated ammonium sprayed directly onto sediment surfaces. It therefore appears that either BMA do not make great use of water column ammonium or that their use of it is through more complicated pathways in the sediments.

Ammonium concentrations in the overlying water *in situ* averaged from 5-13 μmol/L, but those in the sediments were far higher. For example, at 2 cm depth, ammonium reached up to around 180 μmol/L. There was a clear gradient of ammonium flux from the sediment to the sediment-water interface, which supplied BMA with a ready source of nitrogen for growth in habitats they occupied in the surface sediments (Lohrer et al. 2010). Outward flux from the sediment was demonstrated in the laboratory experiment, where ammonium concentrations increased in the source water buckets during the first three days of the experiment. The decrease in the ammonium concentrations after this indicated that either BMA were required

to use water column nutrients once the sediment nutrients had diffused out, or that ammonium was being sequestered in the sediments. The actual usage of the nitrogen for BMA assimilation, as well as sediment-water exchange, was indicated by the lower values of nitrogen at the sediment surface, compared to greater depths (Liu et al. 2003, Longphuirt et al. 2009) and by the negative correlation between ammonium at the sediment surface and BMA biomass, suggesting that BMA were using up the nitrogen.

Ammonium concentration in new and old sediments seems to be complicated. There was clearly high variance associated with the concentrations of ammonium, as would be expected in a field setting. Old sediments had an average of up to 30% greater ammonium deeper down than did new sediments. This is probably a reflection of the old sediments being exposed to eutrophication for several decades and perhaps that convex surface features, such as the new sediment mounds, are more prone to hydrodynamic stress and export of organic matter and nutrients (Sogard & Able 1991, Huettel et al. 1996). The quick accumulation of ammonium in new sediments, which are relatively porous due to their coarse grain size, suggests that there were high levels of nutrients and organic matter being supplied to the sediments from the overlying water column following the emergence of the sediments in 2011. The high concentrations in both sediment types, compared to the overlying water, meant that the diffusion gradient of ammonium would be from sediments to overlying water, and is indicative that a nitrogen supply from the overlying water column to BMA in the sediments would not be favoured. Consequently, nutrients from the sediment are more readily available to BMA than nutrients from the overlying water column and BMA are, therefore, likely using porewater nutrients more than nutrients in the overlying water.

The relatively constant ammonium concentrations in the sediments between weeks indicated that ammonium was being produced, most likely by bacterial breakdown of old organic matter (Graham & Wilcox 2000), in high enough rates to outweigh the consumption of ammonium by BMA, and that the diffusion rate out of the sediments was slow (Krom & Berner 1980). Low diffusion rates paired with high production rates of ammonium could create a large supply of porewater ammonium for BMA to feed on for long periods of time.

Several other studies in which nutrients have been supplied to the water column and directly to the sediment surface found similar results and concluded that nutrients are not limiting to BMA biomass or primary productivity. This is due to the high background levels of nutrients found in many estuaries and the release of nutrients from the sediments (Montgomery et al.

1979, Welsh 1980, Underwood et al. 1998, Armitage et al. 2009, Piehler et al. 2010). Even in coarse sediments, with high porosity and a quick uptake of water column nutrients, BMA appear to be buffered against changes in overlying nutrients because they are using sediment nutrients (Nilsson & Sundback 1991). Similar to the results in my laboratory study, Piehler et al. (2010) found that although there were increases in mean BMA biomass in response to raising water column nutrient levels in sand flats, the variability within treatments was so high that there was no statistical significance among treatments. The sources of variability in my experiments could have been an artefact of laboratory conditions, such as the uneven irradiance level across the experimental setup (200-300 µmol m^{-2} s^{-1}), although replicates were randomly placed. There could have also been differences because of an uneven distribution of BMA at the start of the experiment, although I took reasonable measure to ensure this would not be the case. Chlorophyll *a* levels in the source water buckets indicated that small numbers BMA were moving on or off of sediment cores, but this was unlikely to cause high variability among treatments. More likely, small differences in the BMA community and microscale differences in surface sediment composition were the sources of variability within treatments. Although there were visible differences between old and new sediments, the grain size in the surface layer was a composite of small to large grains. Therefore, although whole communities on old and new sediments may differ, at the scale of these experiments, those differences may be obscured.

Because fertilization had little overall effect on BMA biomass or productivity, it is likely that BMA are either growing and colonising to maximum efficiency, or that they have reached maximum biomass and are being limited by other factors (Graneli & Sundback 1985). Although within the context of my experiments, sediment type accounted for 21% of the variation in chl *a*, significant negative correlations among several variables suggested the importance of several other factors in regulating BMA biomass. These included macroalgal cover, *A. crenata* abundance, and infaunal biomass. In at least the case of *A. crenata*, the negative relationship between its abundance and the level of chl *a* was significant only in the new sediments, suggesting an interactive effect between major grazers and sediment type. These relationships were in accordance with results from my other experiments, and the mechanisms behind them are discussed in Chapter 4. In particular, the greater abundance of invertebrates and macroalgae on old sediments, and their effects, are explored. The lack of effect of fertilization on grazing invertebrate densities seen in this study was also noted by Armitage et al. (2009). The same study also found that the grazing effect on BMA biomass

was so strong and background nutrient levels were so high, that they obscured any effects of nutrients on BMA. The stronger effects of macrograzers, compared to nutrients, as regulators of BMA has also been noted in other estuaries (Lever & Valiela 2005, Armitage et al. 2009).

Although the moderately strong relationship between chl *a* and primary production, as found in old sediments, has been indicated before (Colijn & Dejonge 1984), the absence of a relationship between chl *a* and primary production in new sediments is difficult to explain, but could be due to the measurement of non-actively photosynthesizing BMA biomass found at depths at which the contact core measured (Pinckney & Zingmark 1993). That production values were higher in the old sediments in week 3 was surprising, as chl *a* levels were higher in the new sediments during this time. Many studies have correlated BMA biomass with production and found r^2 values ranging from 0.08 to 0.55 (Cadee & Hegeman 1974, Davis & McIntire 1983, Colijn & Dejonge 1984, Grant 1986, Wasmund 1986, Sullivan & Moncreiff 1988), indicating that the relationship between chl *a* and biomass is not always linear or predictable. It is possible that differences in light regime and other covarying factors, such as such as sediment stability and organic content (Admiraal et al. 1982, Peletier 1996), were responsible for the higher production values seen in the old sediments. Light intensity reached saturation levels for at least the first two weeks of the experiment, but hardware problems prevented the transfer of light and temperature data for the final two sampling periods, and it is possible that light was limiting NPP in new sediments at this time.

Although BMA species diversity can be altered by changes in nutrient regime in some cases (Peletier 1996), this likely did not occur in my study because analyses determined that biomass, production and sediment nutrient levels were not significantly affected by fertilizing treatment.

3.4.1 Summary

From this study, it is clear that ecosystems do not necessarily respond linearly to changes in nutrient loads. The AHE is highly eutrophied, and organic matter and nutrients have been sequestered in the sediment, providing an available source of nutrients for growth to BMA. They are, therefore, no longer limited by nutrients but may be limited by other environmental factors, such as grazing. The high abundance of BMA across sediment types, and their ability to use nutrients from within the sediment column, imply that they could be an important

buffer against eutrophication and could, alongside the wastewater diversion, help return the estuary to a non-eutrophied state. The large amounts of ammonium sequestered in the deeper sediments, particularly in the old sediments, and the addition of organics and nutrients to the estuary following the earthquakes may have prolonged the recovery process. However, the appearance of the new sediments does not seem to have had a detrimental effect; new sediments have a lower pool of ammonium than old sediments, and BMA primary production does not differ between sediment types.

CHAPTER 4

The effects of earthquake-driven sediment change on biotic interactions in a benthic estuarine environment

4.1 INTRODUCTION

The functioning of a healthy estuarine ecosystem depends on complex interactions between the physical, chemical and biological processes within it (Godbold et al. 2011). Intertidal flats are characterised by large amounts of biological activity (Little 2000), and there are often subtle interactions between the flora and fauna within them (Owen 1992). For example, through primary production, benthic microalgae (BMA) alter the supply of oxygen, nutrients and organic matter to the sediment (Little 2000, Corzo et al. 2009). This source of organic matter is the food base for macrobenthic fauna, which are capable of attaining considerable biomass on estuarine flats (Little 2000). By grazing on BMA and distributing gases and nutrients throughout the sediment, in a process called bioturbation, macrobenthic organisms are, in turn, responsible for the distribution and abundance of BMA though direct and indirect interactions (Andersen & Kristensen 1988, Little 2000, Stocks & Grassle 2001). The growth of macroalgal species can also be fuelled by bioturbation, and as macroalgae accumulate they can alter the BMA and benthic faunal communities through indirect interactions such as competition for resources and habitat modification (Andersen & Kristensen 1988, Sundback et al. 1996, Hansen & Kristensen 1998).

The Canterbury earthquakes of 2010 and 2011 were cataclysmic disturbance events that resulted in large amounts of pristine sediments being deposited on the floor of the estuary. Some of the earthquake-induced changes in the physical and chemical interactions with BMA were examined in previous chapters, but there is also a suite of biological interactions, both direct (invertebrate grazing) and indirect (competition and bioturbation), that could have been altered as well. Understanding the relative influence of direct and indirect influences on BMA

in the altered estuary will help us gain a better understanding and more robust predictions about how interaction dynamics and recovery processes within the ecosystem have changed.

4.1.1 Direct and indirect effects of macroinvertebrates on benthic microalgal communities

Invertebrates can exert major trophic influences on sediment associated microalgal communities in estuaries (Hagerthey et al. 2002) by modifying BMA distribution and abundance (Knox 1992). There are many groups of macroinvertebrates in the Avon-Heathcote Estuary (AHE), with gastropods, crustaceans and polychaetes among the most common and well-studied (Jones 1983, Juniper 1987, Griffin & Thomson 1992). These groups commonly exhibit deposit or filter feeding behaviours (Owen 1992) and depend on microalgae as a major carbon source. Accordingly, several studies have shown that feeding by invertebrates directly limits microalgal standing stock (Levinton 1985, Juniper 1987, Stocks & Grassle 2001).

Invertebrates can also alter the distribution and abundance of BMA communities indirectly through disruption and mixing of the sediments in a process called bioturbation (Biles et al. 2002, Needham et al. 2010, Beauchard et al. 2012). Although the disruption of sediments by invertebrates can make them more prone to resuspension and erosion, it can also distribute porewater, organic matter and oxygen throughout them, with the depth and intensity of mixing being species specific and related to movement patterns and burrow type (Knox 1992, Hansen & Kristensen 1997, Stocks & Grassle 2001, Gerino et al. 2003, Orvain et al. 2004, Needham et al. 2010). The ventilation activities of the organisms in the burrows can enhance the exchange of solutes across the sediment-water interface, while the release of metabolites, such as ammonia, is mediated by the stimulation of mineralisation processes in the burrow wall (Andersen & Kristensen 1988, Hansen & Kristensen 1998, Corzo et al. 2009, Needham et al. 2010). Microalgae living at the sediment surface can intercept nutrients, such as ammonia, as they move across the sediment-water interface (Beauchard et al. 2012), and since the release of ammonia enhances primary production by BMA (Biles et al. 2002), bioturbating invertebrates provide an important ecosystem function in estuaries (Needham et al. 2010, Beauchard et al. 2012). Accordingly, some studies have suggested that indirect effects on BMA communities may be equally, if not more, important than direct trophic impacts (Meadows & Tufail 1986, Needham et al. 2010).

4.1.2 Macroalgal mats as indirect regulators of benthic microalgae

Macroalgae are natural components of estuarine soft sediment communities (Knox 1992), and various species, particularly *Ulva lactuca*, have been found drifting in most sites in the AHE (Bressington 2003, Bolton-Ritchie 2011a). *Ulva* is a genus with many morphotypes and although it has been found that *Ulva lactuca* is one of the main species found in the AHE (Knox & Kilner 1973), the taxonomy is currently unresolved and this species will be referred to as *Ulva* sp. for the duration of this chapter. With their high surface area to volume ratio, the thalli of *Ulva* sp. are able to exhibit high nutrient uptake (Rivers & Peckol 1995) and, consequently, *Ulva* sp. has become a major primary producer in the AHE, where it is able to grow and reproduce extensively under eutrophic conditions (Bressington 2003). Furthermore, this early coloniser is opportunistic and able to grow rapidly after a disturbance (Rivers & Peckol 1995), indirectly affecting BMA communities though competition for resources, such as nutrients and light (Knox 1992, Sundback et al. 1996, Bolton-Ritchie 2011a). Other ecosystem functions can be negatively affected when respiration and decomposition of these macroalgal mats create anoxic conditions in the sediment and water, causing mortality of benthic fauna (Andersen & Kristensen 1988, Hansen & Kristensen 1998).

4.1.3 Potential effects of earthquake disturbance on benthic interactions

It is possible for disturbance events to alter direct and indirect influences in ecosystems, especially when habitat structure is altered (Godbold et al. 2011). Through changes in sediment composition, the Canterbury earthquakes could act to alter ecosystem functioning in a variety of ways. Hydrological patterns, nutrient levels and organic content can differ between coarse and fine sediments (Huettel et al. 1996, Lever & Valiela 2005), which could influence the location, attachment and rate at which macroalgal species grow (Huettel et al. 1996, Nedwell et al. 2002, Anibal et al. 2007), potentially altering competitive dynamics with BMA. Furthermore, the impact of invertebrates on BMA is likely to be related to their density, feeding behaviour, burrow morphology and burrow permanency, all of which can vary among sediment types (Tsuchiya & Kurihara 1979, Needham et al. 2010). Many species prefer fine, muddy sediments (Barnes & Greenwood 1978, Tsuchiya & Kurihara 1979), because of the large amount of microalgae, nitrogen and organic material found within them (Little 2000, Mann 2000). The preference for muddy sediments is also dictated by the ease of bioturbation activities such as burrowing, as well as an upper limit to the size of sediment that

can be ingested when grazing (Tsuchiya & Kurihara 1979). If the grazing and bioturbation activities of species are altered due to the emergence of the new sediments, this could lead to changes in BMA biomass and ecosystem functioning (Sundback et al. 1996, Gerino et al. 2003, Orvain et al. 2004, Needham et al. 2010, Beauchard et al. 2012). Godbold et al. (2011) demonstrated that bioturbation intensity was altered in response to resource heterogeneity and that small scale variations in habitat structure influenced the contribution of different species to the ecosystem. Sundback et al (1996) found that invertebrate grazing activity was also changed in response to sediment type, with invertebrate trophic interactions limiting BMA biomass in subtidal sediments, but not in sandy coastal sediments. As large sized bioturbators tend to be slow growing and long lived, their effect on BMA biomass could be long lasting, and the recovery of ecological functioning following large scale disturbance to benthic communities could be constrained by the changes in direct and indirect interactions with invertebrates (Beauchard et al. 2012).

Studies of interactions between primary producers and biotic forces may clarify the regulating mechanisms of BMA, improving our insight into how the physical properties of the sediment have altered the ecosystem. Here, I examine the direct and indirect interactions between BMA and invertebrates and macroalgae between two different sediment types.

4.1.4 The use of phaeophytin *a* as a marker of invertebrate grazing

Chlorophyll *a* (chl *a*) is rapidly turned into phaeophytin *a* through the loss of a magnesium group in the digestive system of benthic invertebrate grazers (Abeleoeschger & Theede 1991, Buffan-Dubau et al. 1996). Phaeophytin *a* can be detected, by pigment analysis, in animal faeces and in marine and freshwater sediments (Abeleoeschger & Theede 1991) and, therefore, these pigments have been used as indicators of invertebrate grazing intensity on BMA (Boyd et al. 1980, Cartaxana et al. 2003).

Many researchers caution against the use of phaeophytin *a* as a proxy for invertebrate grazing because in variable cases chl *a* can be degraded directly into colourless products, rather than phaeophytin *a* (Gieskes et al. 1991, Ford & Honeywill 2002). Still, others have demonstrated a close match between the appearance of phaeopigments and the disappearance of chl *a*, and maintain that it is acceptable to consider phaeophytin *a* as a marker of invertebrate grazing intensity, as long as chl *a* is considered in conjunction with it (Boyd et al. 1980, Cartaxana et

al. 2003). Consequently, the phaeophytin *a:* chlorphyll *a* ratio has been used as an indicator of the condition of microalgal populations, with sediments that have been grazed upon by invertebrates generally having significantly higher ratios than those that have not been grazed upon (Cartaxana et al. 2003). Phaeophytin *a* analysis was used here to further examine the mechanisms controlling BMA biomass on different sediment types, and to further investigate if it is a useful marker of invertebrate grazing intensity in estuaries.

4.1.5 Hypotheses

In the months preceding the February and June earthquakes, monthly sampling showed microalgal biomass to be dynamic and generally lower on new sediments than on old sediments (Section 2.3.1). It also appeared that macroinvertebrate fauna were less common on new sediments. It was postulated that this could be due an inadequate amount of microalgal food resources to support grazing on new sediments, or the inability of the fauna to graze on coarse grained sediments (Little 2000).

This chapter is split into three main sections. The first section describes the direct trophic effects of an important invertebrate on BMA biomass and recolonisation. The mudflat snail, *Amphibola crenata*, was chosen for these studies as it is one of the most common invertebrates in the AHE (Owen 1992, Bolton-Ritchie 2011a) and has been shown to graze on microorganisms and organic matter in the sediment (Juniper 1982, 1987). The functionally similar gastropod, *Hydrobia ulvae*, can only ingest sand particles between 20 and 200-300 µm (Barnes & Greenwood 1978), and if *A. crenata* has similar restraints on the size of particle it can ingest, it would be expected that grazing would be limited to old sediments, where grain sizes were generally smaller (Section 2.3.1). It was, therefore, hypothesised that *A. crenata* would not be able to graze on BMA in new sediments, and that an inverse relationship between *A. crenata* density and BMA biomass would only be seen on old sediments, where grazing was possible. It was predicted that as *A. crenata* density increased, the recolonisation capabilities of BMA would be decreased, but only in old sediments where *A. crenata* was able to graze. This section also investigates the use of phaeophytin *a* as an indicator of invertebrate grazing intensity. Based on the results from previous studies, it was hypothesised that the phaeophytin *a*: chlorophyll *a* ratio would be a good indicator of macroinvertebrate grazing. High ratios would reflect the presence of intense grazing activity, as well as an increase in the density of invertebrate grazers. The ratio would also help to

examine if negative effects on BMA were due mostly to grazing (an increased ratio) or bioturbation (a decreased ratio).

The second section explores the indirect effects of a variety of benthic invertebrate functional groups, defined by their bioturbation activities, on microalgal biomass. The polychaete, *Nicon aestuariensis*, forms permanent burrows up to 40cm deep (Owen 1992), and the brachyuran mudcrab, *Macropthalmus hirtipes*, constructs temporary burrows primarily for protection from predators (Jones 1983). *Macropthalmus hirtipes* does not depend on its burrow for respiration, feeding or cooling (Jones 1983). *Amphibola crenata* thoroughly disrupts the top layer of sediment as it moves and feeds, and burrows just prior to the incoming tide to prevent itself from dislodging from the sediment (Bennington 1979). It then emerges during low tide to graze on surface sediments (Jones 1983). It was hypothesised that the coarse, unstable nature of the new sediments would make them unsuitable for grazing and burrow building by any of the invertebrates, resulting in a lack of significant effect of invertebrates on BMA biomass. It was thought that in old sediments, the extensive burrow matrix built by polychaetes would increase BMA biomass while the temporary burrows of the mud crab would decrease BMA biomass, due to constant reworking and destabilization of the sediment surface.

The final section shows the indirect effects of macroalgal cover on BMA biomass plots in patches where new and old sediments have mixed together. The effect of *Ulva* sp. percent cover and thickness on BMA biomass was determined during an *in situ* experiment. It was hypothesised that *Ulva* sp. mats would be able to dramatically reduce BMA biomass, predominantly due to a reduction in light underneath the mats, but also a result of decreases in sediment organic content and a build-up of sediment around the mat. These effects would become more prominent with increasing *Ulva* sp. thickness and percent cover.

4.2 METHODOLOGY

4.2.1 Methodology pertaining to laboratory investigations

Humphreys Drive (Figure 1.1) was chosen for core gathering for both laboratory experiments in this chapter (Sections 4.2.2.1, 4.2.3), primarily because there were few infauna, which could have potentially affected chl *a* concentrations during the experiments.

Random 1 x 1 m plots were selected from three patches with old sediment and three patches with new sediment. These were usually quite visible as "mounds" (new sediments) and "flats" (old sediments). All plots were located in the mid-tidal level at Humphreys Drive and were at least 2 m away from each other. Polyvinyl chloride (PVC) piping, with an internal diameter and length of 8 cm, were used to take three or four sediment cores from within each plot, the number depending on the experiment (see below). Cores from new sediments were taken from the tops of the sediment mounds. The core pipes were inserted 4 cm into the sediment. A hand trowel was used to dig the cores from the sediment and PVC caps were then placed on the bottom of each core. Cores were placed together in a plastic bin for immediate transport to a temperature controlled laboratory, set at 15°C (±2°C). The top 1 cm of sediment was scraped off of each core, mixed together in a container, and then microalgae were reinfused to obtain an even spread over treatments (see Appendix 1 for scraping and reinfusing methods).

Invertebrates (*A. crenata*, *M. hirtipes*, and *N. aestuariensis*) were collected the next day (day 1) from the Heathcote River Mouth site (Figure 1.1). The Heathcote River Mouth site was chosen because it is the closest site to Humphreys Drive that is inhabited by invertebrates (about 500 m away). The invertebrates were taken to the laboratory in a bucket containing estuary mud and surface water. They were then placed on sediment cores immediately and, to prevent them from escaping, strawberry mesh (2 x 2 cm holes) was placed over the top of the cores and secured in place using a sterile rubber band (Figure 4.1). Cores were placed into a tidal flow-through system, and light and tidal cycles were initiated (16 L: 8 D and 6.25 h immersion: 6.25 h emersion; see Appendix 1 for details on the tidal-flow through system). Water for these experiments was collected from the AHE at Beachville Road and filtered for 24 hours prior to use, first through a 20 µm pleated polypropylene filter, then through a 5 µm polypropylene filter and finally through a 0.9 µm ceramic filter. Water in each of the buckets was changed every day and cores were checked to ensure that invertebrates were alive and had not escaped.

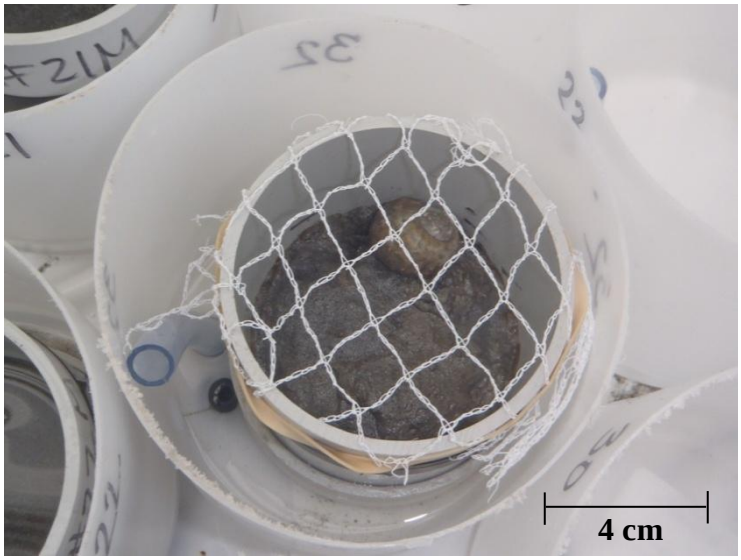

Figure 4.1: Photograph showing a fully assembled experimental laboratory core (50.3 cm^2) containing one *Amphibola crenata.*

On day 5, invertebrates were removed and a contact core (see Appendix 1 for contract coring method) was taken from each sediment core to assess BMA biomass and phaeophytin *a* concentration. A 500 µm mesh bag was used to sieve each core for infauna and any individuals found were preserved in 70% ethanol. In the laboratory, samples were placed in a Bogorov tray with ethanol and searched for animals using a LEICA DC 100 dissecting microscope. The total invertebrate weight was recorded for each core.

4.2.2 Direct effects of macroinvertebrates on benthic microalgal communities

4.2.2.1 Laboratory investigation of the direct effects of macroinvertebrates on benthic microalgal communities

The basic design of this laboratory experiment is illustrated in Table 4.1. Eighteen cores, 9 from new sediments and 9 from old sediments, were collected and prepared as in section 4.2.1, on December 7, 2011 (day 0). On day 1, a field balance was used to determine the wet weight of several *A. crenata* (mudflat snail) in the estuary and 18 individuals, weighing between 5 and 6 g each, were selected and brought back to the laboratory. Three density treatments of *A crenata* were established (0, 1, and 2 per 50.3 cm^2 core), with three replicates on each sediment type. A random number generator was used to determine the position of individual cores within the experimental setup.

Table 4.1: The basic design of the laboratory investigation of the direct effects of macroinvertebrates on benthic microalgal communities, showing (a) the factors included in the design and (b) the sampling procedures that were carried out on each day.

(a) Factors

Sediment Type	*Amphibola crenata* Density	Replicates
New sediment	0	3
	1	3
	2	3
Old sediment	0	3
	1	3
	2	3

(b) Sampling procedure

Day	Procedure
0	Collect sediment cores from Humphreys Drive.
0	Remove top 1 cm of each sediment core.
0	Reinfuse microalgae evenly across cores. Allow to sit overnight.
1	Collect *Amphibola crenata* from Heathcote River Mouth.
1	Place *A. crenata* in laboratory cores and initiate flow through system.
2-4	Check for escaped animals and replace water in source buckets.
5	Take one contact core from each sediment core
5	Sieve cores for infauna

4.2.2.2. In situ examination of the direct effects of macroinvertebrates on benthic microalgal communities

The Heathcote River Mouth site was chosen for this experiment because it has high densities of *A. crenata*. Twenty 1 m^2 quadrats were used to determine the density of *A. crenata* at the Heathcote River Mouth site in the new and old sediments and to find the highest density of *A. crenata* at a variety of sites within the estuary. One hundred *A. crenata* were then collected from random areas within the site and a field balance was used to determine their wet weights. They were then washed, dried at 50°C for 30 hours, and total body dry weights (shells intact) were obtained. The data obtained from this sampling period was used to determine *A. crenata* densities for the experiment, and to back calculate the starting dry weights from wet weights of experimental *A. crenata*.

The experiment was done over 28 days, from November 15 to December 13, 2011. There was a limit on how much time was available to take contact cores during low tide so it was necessary to run the experiment in two parts; new sediments were examined from November 15 until November 29th and old sediments from November 30th through to December 13th. The experiment in the old sediments ran for one day less than it did in the new sediments, due to forecasted poor weather conditions that would have prevented sampling on the final day. Table 4.2 illustrates the experimental design used in this field experiment.

Square grazer cages (625 cm^2) were used to enclose *A. crenata* over the course of the experiment. Each cage was made from four 18 x 25 cm strips of plastic gutter guard (12 x 12 mm holes), cable tied together at the short ends to form a square. A 35 cm long bamboo stake was fastened to the inside of each corner with cable ties and the bottom 15 cm of the bamboo sticks were positioned to stick out of the bottom of the cage.

Eight cages were placed in each of four patches of old and new sediments at the mid tidal level. To aid in the insertion of the cages into the mud, a 25 cm wide metal square box corer (30 cm deep) was inserted fully into the sediment and a shovel was used to gently pull the sediment outside the corer away from each side (Figure 4.2a). The frame was removed, leaving a cage sized area intact and undisturbed, but allowing cages to be inserted into the sediment (Figure 4.2b). Cages were pushed down by their bamboo corners until there was 10 cm of cage remaining on the surface (Figure 4.2b). Cages were located at least 1.5 sampling units away from each other. Four bamboo sticks were used to mark the corners of two 25 x 25 cm plots, which represented control areas (Figure 4.2c). During cage placement, sediment within the cages was minimally disturbed, but was also allowed to settle overnight before sampling began.

Table 4.2: The basic design of the *in situ* investigation of the direct effects of macroinvertebrates on benthic microalgal communities, showing (a) the factors included in the design and (b) the sampling procedures that were carried out on each day. The experiment ran for 14 days in the new sediments and 13 days in the old sediments.

(a) Factors

Sediment Type	Scraping Treatment	*Amphibola crenata* Density	Replicates
New sediment	Scraped	0	4
		4	4
		8	4
		12	4
		control	4
	Non-scraped	0	4
		4	4
		8	4
		12	4
		control	4
Old sediment	Scraped	0	4
		4	4
		8	4
		12	4
		control	4
	Non-scraped	0	4
		4	4
		8	4
		12	4
		control	4

(b) Sampling procedure

Day	Activity
Prior to experiment	Place cages in sediments at Heathcote River Mouth.
0	Take three contact cores from inside every cage.
0	Scrape off top 1 cm of sediment in half of cages.
0	Take three contact cores from inside scraped cages.
0	Collect, weigh, label *Amphibola crenata* from Heathcote River Mouth.
2-12	Remove debris from outside of cages.
13	Check for escaped animals and replace water in source buckets.
13	Take three contact cores from inside every cage.
13	Search sediment for infauna.

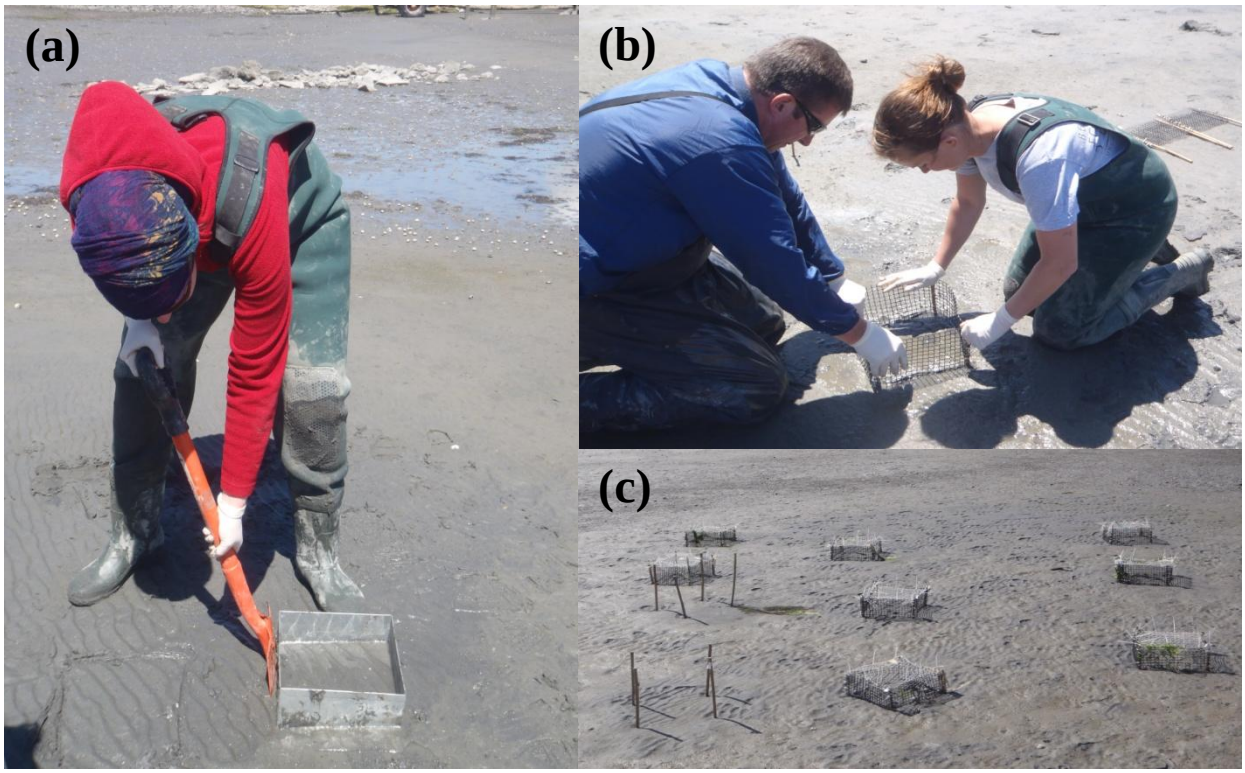

Figure 4.2: Photographs depicting (a) the use of a shovel to pull away the sediment from the outside of the metal frame, leaving the interior undisturbed, (b) insertion of a grazer cage into the square shaped cutout left by the metal frame, (c) and a replicate of grazer cages on new sediments. Cages are shown on day 2 of the experiment, with mesh secured on top.

All plots were sampled twice, once each on the first (day 0) and final (day 13 or 14) days. This was done by taking three contact cores from each cage and control plot to determine BMA biomass and phaeophytin *a:* chlorophyll *a* ratios (see Appendix 1 for methods for processing chl *a* samples). A random number generator was used prior to the experiment to determine the positions of contact cores within the plots, and grids of each cage were drawn and marked to ensure that sampling did not occur in the same place during sampling on the final day of the experiment. To test for the effects of *A. crenata* density on microalgae recolonisation, the top layer of sediment was removed from half of the cages and controls; a ruler was inserted into the ground and a paint scraper was used to remove 1cm of sediment. Cages that never had the top 1cm of sediment removed are referred to here as "non-scraped" treatments, while those that did have the top layer of sediment removed will be referred to as pre- and post-scraped treatments, depending on whether data is referring to samples taken before scraping or after scraping.. Although it was possible that scraping would not remove

all microalgae in the sediments, its primary purpose was to ensure that the sediment surface was not saturated with microalgae, and that recolonisation could occur. Sediment size and composition did not appear to differ between the surface and a depth of 1 cm. Three additional contact cores were taken from plots following scraping, from the same location they had been taken previously, to determine how much BMA biomass had been removed. Sampling occurred over two days due to the lack of time available during a single low tide.

To collect animals for the experiment, 192 *A. crenata* were collected from random areas around the Heathcote River Mouth site after contact coring was done. Each snail was washed in seawater, dried with paper towel, marked with a number using a permanent marker, weighed using a field balance, and then put into one of the cages. Numbering the *A. crenata* made it possible to determine if any had escaped at the end of the experiment and if any new individuals had entered. Wet weights of snails were transformed to dry weights using the regression equation generated in the section above. In each patch of old and new sediment, the cage densities were as follows: 2 replicates of 0, 4, 8, and 12 *A. crenata* per 25 x 25 cm cage. One cage of each density in each replicate patch was scraped and one was unscraped. After all *A. crenata* were placed in the cages, strawberry mesh (2 x 2 cm holes) was cable tied to the top of each cage to prevent other *A. crenata* and macroinvertebrates from entering (Figure 4.2c).

Cages were checked daily to ensure they were not occluded by macroalgae or other debris. The control plots and cages could potentially trap *Ulva* sp., but cages were generally devoid of most material. Any material covering cages and control plots was removed and carried away from the area.

After 14 days (13 in the old sediments), three contact cores were taken from a randomly designated area in each cage, and the sediment was searched for infauna. *Amphibola crenata* were removed from each cage and wrapped in a wet paper towel for transport back to the laboratory. They were dried at 50 °C for 30 hours and their final dry weights were recorded and compared to their initial dry weights to determine weight changes. Dry weights, rather than wet weights, were used to determine weight loss.

4.2.3 Laboratory investigation of the indirect effects of invertebrate functional groups on benthic microalgal communities

Table 4.3 illustrates the basic design of this laboratory experiment. Sediment cores were collected and prepared, as in section 4.2.1, on January 20th, 2012 (day 0).

Table 4.3: The basic design of the laboratory investigation of the indirect effects of invertebrate functional groups on benthic microalgal communities, showing (a) the factors included in the design and (b) the sampling procedures that were carried out on each day.

(a) Factors

Sediment Type	Invertebrate species	Replicates
New sediment	*Nicon aestuariensis*	*3*
	Amphibola crenata	*3*
	Macropthalmus hirtipes	*3*
	Control	*3*
Old sediment	*Nicon aestuariensis*	*3*
	Amphibola crenata	*3*
	Macropthalmus hirtipes	*3*
	Control	*3*

(b) Sampling procedure

Day	Procedure
0	Collect sediment cores from Humphreys Drive.
0	Remove top 1 cm of each sediment core.
0	Reinfuse microalgae evenly across cores. Allow to sit overnight.
1	Collect invertebrates from Heathcote River Mouth.
1	Place invertebrates in laboratory cores and initiate flow through system.
2-4	Check for escaped animals and replace water in source buckets.
5	Take one contact core from each sediment core
5	Sieve cores for infauna

Twelve cores were taken from new sediments and 12 from old sediments. Polychaetes (*Nicon aestuariensis*), mudflat snails (*Amphibola crenata*) and mudcrabs (*Macropthalmus hirtipes*) were collected from the Heathcote River Mouth site on day 1. Once collected, the invertebrates were transported back to the laboratory in separate containers. Three replicate cores were used in each of the four treatments: control (0 invertebrates), 1 x *A. crenata*, 1 x *M. hirtipes*, and 1 x *N. aestuariensis*. A random number generator was used to determine the position of each core within the system.

4.2.4 Macroalgal mats as indirect regulators of benthic microalgae on a mixed sediment substrate

Humphreys Drive was chosen for this experiment because it had been unusually devoid of macroalgal species (see Section 2.2.1) for several months. In January 2012, eighteen 60 x 80 cm plots were marked on the lower mid tidal level at this site. They were placed in patches that visually contained a thorough and even mixture of old and new sediments. Corners were marked using PVC piping (10 mm internal diameter) and plots were located at least one metre away from each other. Only two corners of each plot were marked to decrease the chances that drifting macroalgae would get trapped on plots.

Once plots were established three contact cores were taken from each plot to determine BMA biomass (see Appendix 1 for contact coring methods). One sediment sample was taken per plot for grain size and organic content analysis. The locations of the contact cores and sediment samples were established using a random number generator and grids of each plot were drawn and marked to ensure that sampling did not occur in the same place during sampling on the final day of the experiment.

Large amounts of *Ulva* sp. with wide fronds was collected from Sandy Point (Figure 1.1), stripped of grazers, and brought to experimental plots. Two experiments were set up at this time. One involved manipulating macroalgal percent cover (0, 50, 100%), and the other manipulated macroalgal thickness (control, thin, medium, thick) to test their effects on BMA biomass (Figure 4.3). There were three replicates for each treatment. In some coastal waters, macroalgal mats occur in thicknesses of 5 to 15 cm during the summer months (Dalsgaard 2003). These measurements were used as a guideline, but the light intensity under each mat, measured using a LI-COR (LI-250) light meter, was used to determine the thickness of each treatment and to keep it consistent through time. Thin mats were generally comprised of a single sheet of *Ulva* sp., thick mats were about 5 cm thick and medium mats, and all percent cover treatments, were approximately 2 cm thick.

Blades of *Ulva* sp. was anchored to the benthos using lengths of #8 fencing wire bent into a u-shape. Most of the anchor points were outside of the plots, but occasionally wire had to be within the plots to properly secure the mats. *Ulva* sp. was attached so that parts of it could float upwards on the incoming tide, as it would in natural situations. Any macroalgae that were not used were brought back to the laboratory and kept in a constant flow aquarium tank,

fed with filtered estuary water that had been sequentially filtered through a 20 µm pleated polypropylene filter, a 5 µm polypropylene filter and finally a 0.9 µm ceramic filter.

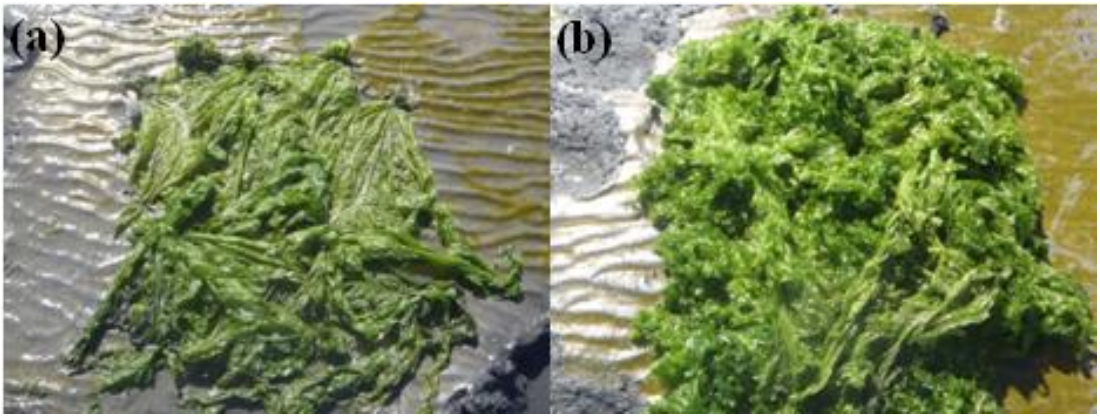

Figure 4.3: Experimental plots (60 x 80 cm) with (a) thin (one sheet) and (b) thick (5 cm) *Ulva* sp. cover.

Plots were checked daily and any extra macroalgae that had washed in with the tide was removed. *Ulva* sp. kept in the laboratory aquarium was brought to the field to replace any experimental *Ulva* sp. that appeared unhealthy, developed holes, or had started to bleach. On day 7, the *Ulva* was carefully removed from the plots, BMA biomass was measured using contact cores, and a sediment sample from each plot was analysed for organic content and sediment grain size (see Appendix 1 for methods of contact coring and processing of sediment samples).

4.2.5 Statistical analyses

4.2.5.1 Laboratory investigation of the direct effects of macroinvertebrates on benthic microalgal communities

The effect of sediment type (old and new) and *A. crenata* density (0, 1, and 2/50.3 cm^2) on chl *a* concentration (µg/g extract) and the phaeophytin *a*: chlorophyll *a* ratio were tested using General Linear Models (GLM). All factors (sediment type and *A. crenata* density) were fixed. A linear regression was used to determine the relationship between chl *a* and phaeophytin *a* concentration.

4.2.5.2 In situ examination of the direct effects of macroinvertebrates on benthic microalgal communities

The amount of chl *a* at the beginning of the experiment was analysed using separate one-way analyses of variances (ANOVA) to determine if the starting biomass was similar between new and old sediments within non-scraped and pre-scraped plots. ANOVAs were also used to determine if BMA biomass was similar between new and old sediments on post-scraped plots and across density treatments within each sediment type (new and old) and scraping treatment (non-scraped and post-scraped). General Linear Models tested for significant effects of *A. crenata* density (0, 4, 8, and 12/625 cm^2), sediment type (old and new), and scraping treatment (scraped or non-scraped surface) on the change in both chl *a* concentration and phaeophytin *a*: chlorophyll *a* ratio. One data point was removed from the analysis of the phaeophytin *a*: chlorophyll *a* ratio because it was greater than 5 standard deviations from the mean and was indicative of sampling error. All factors in the above models were fixed.

A linear regression was used to determine the relationship between wet and dry biomass of the 100 *A. crenata* collected prior to the experiment. The regression equation was used to back calculate the starting wet weights of *A. crenata* to dry weights. A GLM was used to determine if sediment type, *A. crenata* density and scraping treatment had significantly affected the mean dry weight of *A. crenata* per cage at the end of the experiment.

4.2.5.3 Laboratory investigation of the indirect effects of invertebrate functional groups on benthic microalgal communities

GLMs were used to analyse the effect of invertebrate functional group (*A. crenata, M. hirtipes* and *N. aestuariensis*) and sediment type (old and new) on chl *a* concentration and the ratio of phaeophytin *a*: chlorophyll *a*. Both factors (sediment type and invertebrate functional group) were fixed.

4.2.5.4 Macroalgal mats as indirect regulators of benthic microalgae on a mixed sediment substrate

The effect of cover (0, 50, and 100%) or thickness (0, thin, medium, and thick) of macroalgae on sediment grain size, organic content, and chl *a* concentration was determined using GLMs. Organic content and each grain size were converted to percentages of the total sediment sample prior to analysis and a separate GLM was run for each grain size. Macroalgal treatments were fixed factors in all GLMs.

A linear regression was carried out to establish if chl *a* concentrations at the end of the thickness experiment had been affected by light intensity. Because the 50% macroalgal cover treatment was only partially covered by *Ulva* sp., data from the percent cover experiment was not included in the analysis and only the thickness experiment was used to analyse the relationship between light intensity and chl *a*.

For all analyses in this chapter, homogeneity of variances was checked using Cochran's test and when necessary, data were transformed using $\log_{10}$-(x) or $\log_{10}$- (x+1) for count or biomass and arcsin-square root for percentage data. Where this did not help, the significant p-level was reduced from 0.05 to p = 0.01. Subsamples from within individual plots in the macroalgal and *in situ* grazer experiments were treated as random factors, and if differences between them were non-significant (p > 0.25), they were pooled within treatments. Tukey's HSD post hoc tests were used when GLMs indicated significant effects. Data was analysed using STATISTICA 7 and Microsoft Excel 2010.

4.3 RESULTS

4.3.1 Laboratory investigation of the direct effects of macroinvertebrates on benthic microalgal communities

Amphibola crenata appeared healthy throughout the course of the experiment and there were no cases where marked individuals escaped. There were significantly lower concentrations of chl *a* on new sediments compared to old sediments ($F_{1,12}$ = 99.78, p < 0.001; Figure 4.4) and there were significant differences between densities ($F_{2,12}$ = 4.46, p = 0.03), but no interaction between the two factors (p > 0.05). Post hoc tests indicated that cores with a density of 2 *A. crenata* had significantly less chl *a* than those without *A. crenata* (Tukey HSD; Figure 4.4). A total of 82 % of the variation in chl *a* concentration was accounted for by sediment type, and only 7% by *A. crenata* density.

Linear regression showed that there was a significant relationship between chl *a* and phaeophytin *a* concentration (R^2 = 48%, p = 0.001; Figure 4.5). However, the ratio of phaeophytin *a*: chlorophyll *a* was not significantly different between old and new sediments ($F_{1,12}$ = 0.004, p = 0.90; Figure 4.6), mainly because all the values for new sediments were clustered near zero.

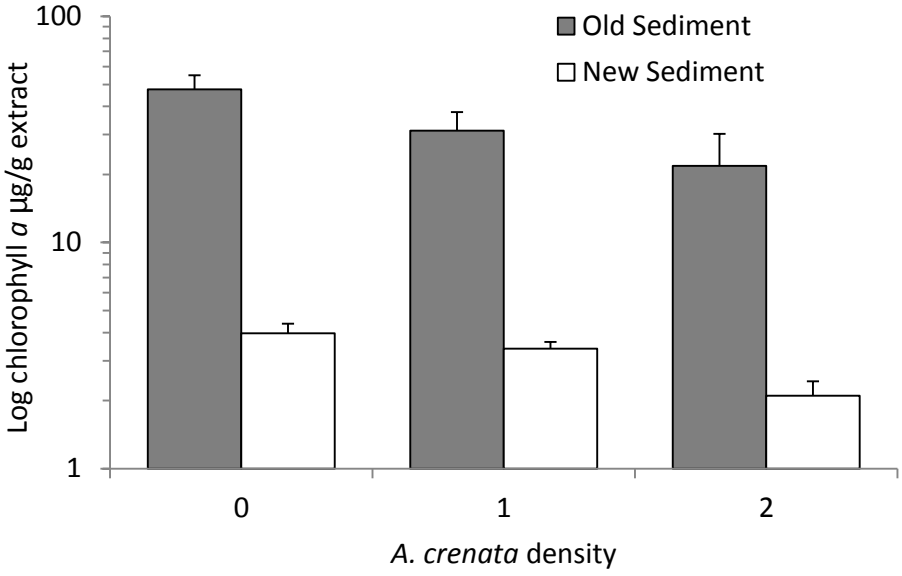

Figure 4.4: Mean (+SE) log chlorophyll *a* concentration in cores (50.3 cm^2) across old and new sediments and *Amphibola crenata* densities (0, 1, and 2) in the laboratory.

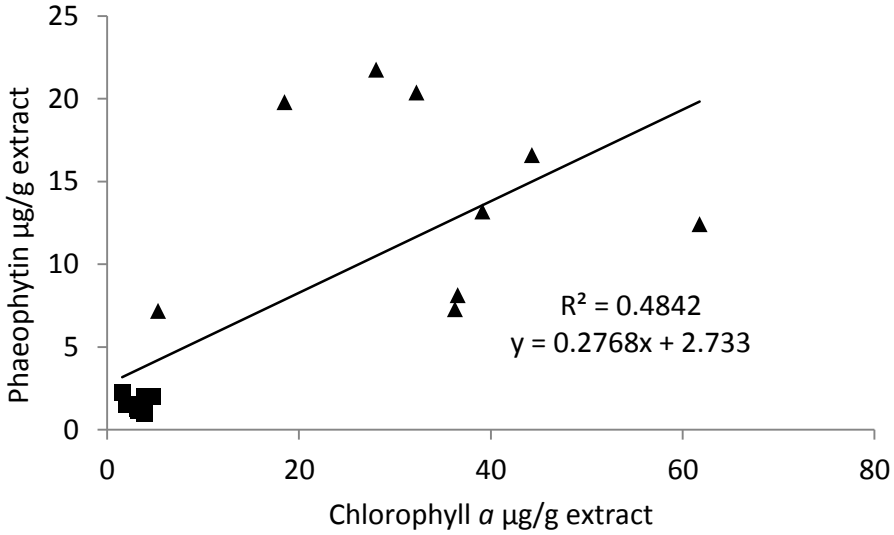

Figure 4.5: Mean phaeophytin *a* (µg/g extract) versus chlorophyll *a* concentration (µg/g extract) for all sediment types (new and old) and *Amphibola crenata* densities (0, 1, and 2/50.3 cm^2). Triangles represent old sediments and squares represent new sediments, n =18.

As for chl *a* concentrations, *A. crenata* density had a significant effect on the ratio of phaeophytin *a:* chlorophyll *a* ($F_{2,12}$ = 5.96, p = 0.01; Figure 4.6) and post hoc Tukey's HSD tests showed that the 2 x *A. crenata* treatment significantly increased the ratio when compared to the no *A. crenata* treatment (p = 0.01). In this case, there was no effect of sediment type ($F_{1,12}$ = 0.005, p > 0.05). Only 49% of the variation in phaeophytin *a:* chlorophyll *a* ratio could be attributed to *A. crenata* density.

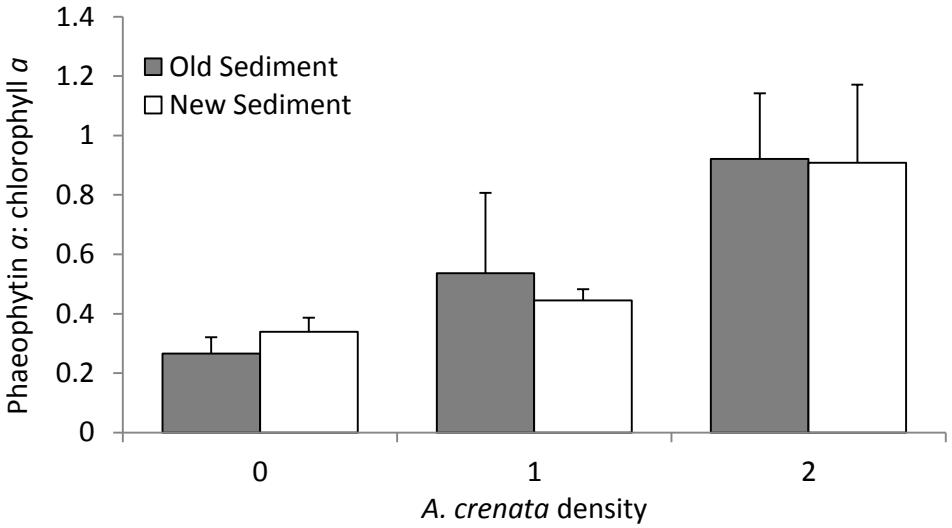

Figure 4.6: Mean (+SE) ratios of phaeophytin *a*: chlorophyll *a* in new and old sediments, across *Amphibola crenata* densities (0, 1 and 2/50.3 cm^2) in the laboratory after 5 days.

It is unlikely that the analyses for this experiment would have been affected by infauna within the sediment cores because, as identified by sieved samples, only two of the eighteen cores contained small polychaetes (< 1 cm long) with a biomass of less than 0.009 g in each core.

4.3.2 *In situ* examination of the direct effects of macroinvertebrates on benthic microalgal communities

The mean (±SE) number of *A. crenata* at the Heathcote River Mouth site, before the experiment (Dec 2011), was 3.3/0.25 m^2 (±0.59) in the old sediments and 1.65/0.25 m^2 (±0.29) in the new sediments with a maximum of 8/0.25 m^2 and 4/0.25 m^2, respectively. Throughout the estuary, the maximum abundance of *A. crenata* found was 11/0.25 m^2 on old

sediments at Plover Street. These data were used to determine appropriate range of *A. crenata* densities for this experiment. Densities of 4 and 8 per 0.25 m^2 were chosen because they were the maximum densities seen in each of the old and new sediments at the Heathcote River Mouth and 12/0.25 m^2 was chosen as the maximum density.

Scraping of the sediment at the beginning of the experiment resulted in a mean reduction of 63% of the chl *a* in the new sediments, and 20% in the old sediments (Figure 4.7). Before scraping, chl *a* was significantly higher in new sediments than in old sediments ($F_{1, 118}$ = 96.69, $p < 0.001$; Figure 4.7). After scraping, there was no longer a significant difference in starting amounts of chl *a* between sediment types ($p > 0.05$; Figures 4.7, 4.8).

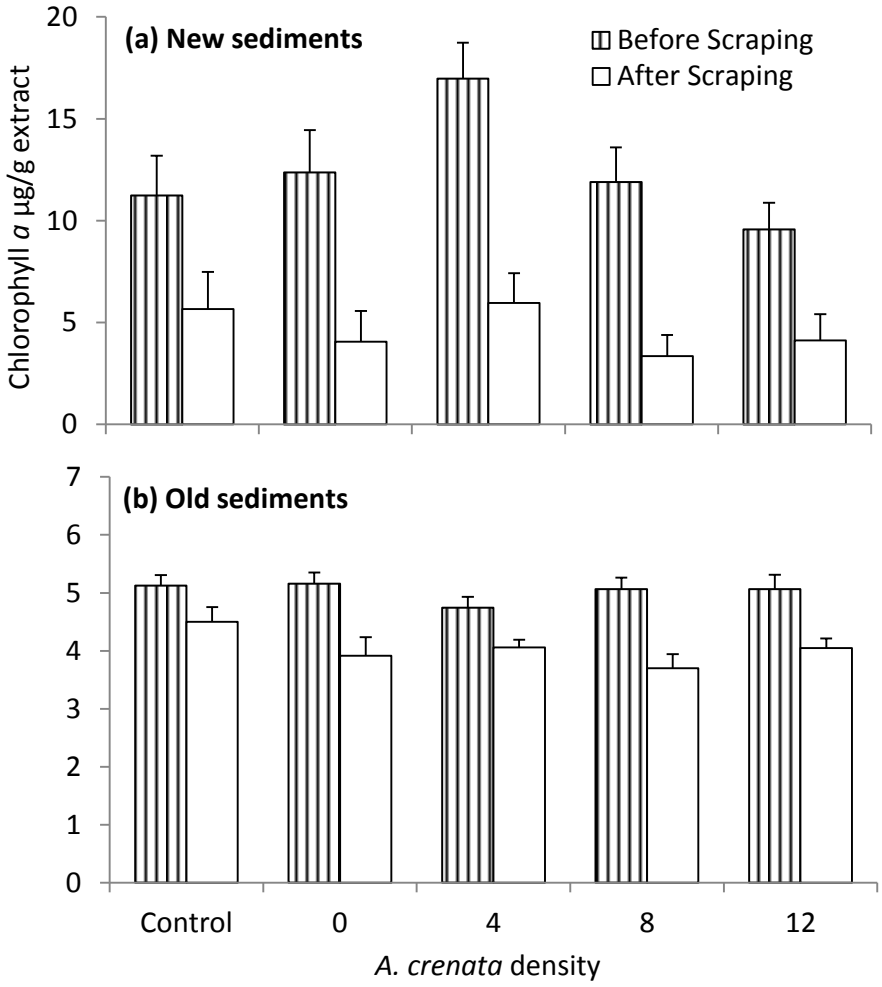

Figure 4.7: Mean (+SE) chlorophyll *a* (µg/g extract) before and after scraping treatment in (a) new sediments and (b) old sediments on day 0. Note change in y axis scale between a and b, n = 12 in each bar.

In sediments that were never scraped (non-scraped treatments), chl *a* was significantly higher in new sediments compared to old sediments ($F_{1, 118} = 84.45$; Figure 4.8). Within all sediment types and scraping types, chl *a* was not significantly different between density treatments ($p > 0.05$; Figures 4.7, 4.8).

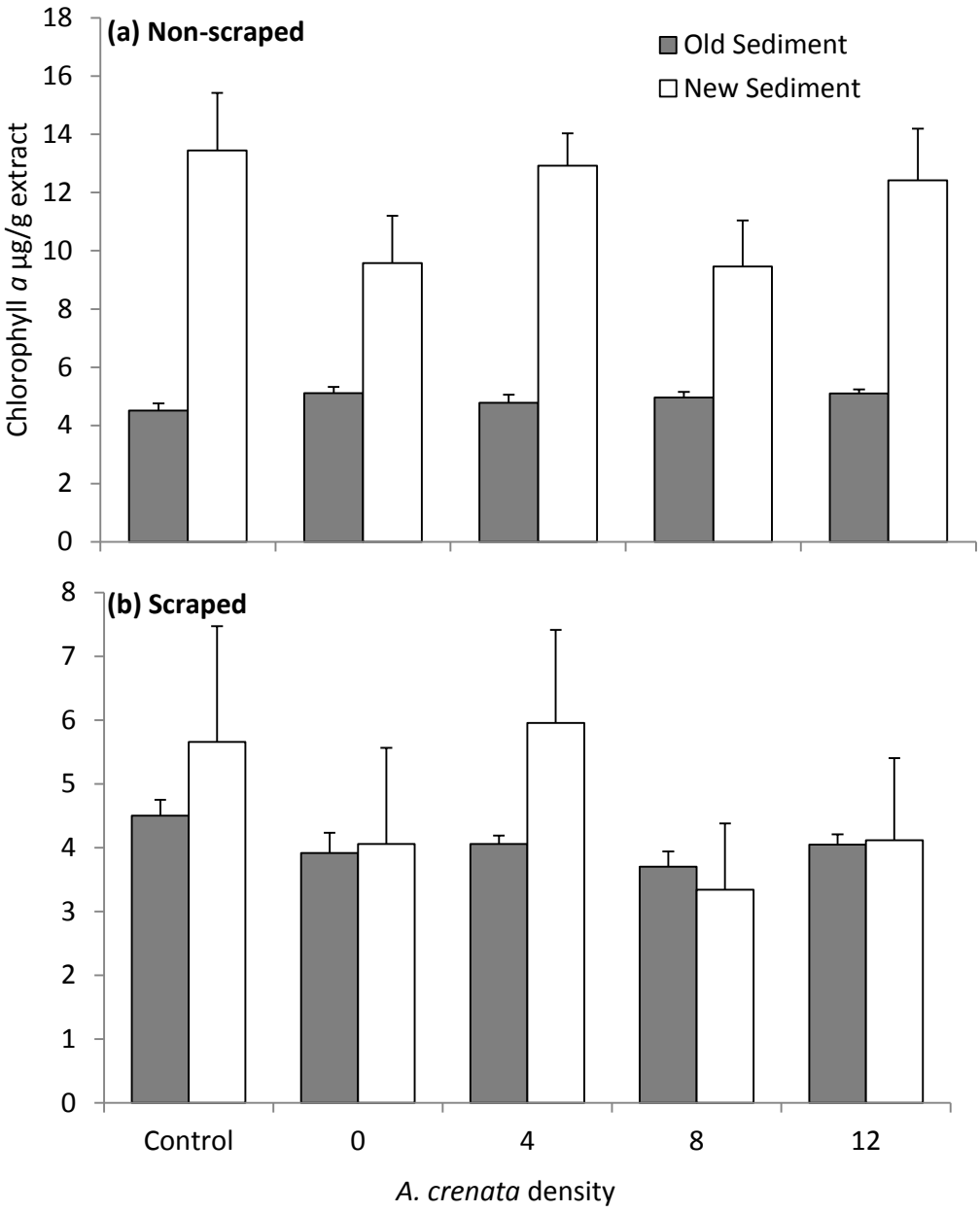

Figure 4.8: Mean (+SE) amount of chlorophyll *a* (µg/g extract) on day 0 in (a) non-scraped and (b) scraped treatments. Note change in y axis scale between a and b.

The mean change in chl *a* over the course of the study was significantly affected by the interaction between sediment type and scraping treatment ($F_{4,220} = 53.77$, $p < 0.001$; Figure

4.9; Table 4.4a). Within this effect, Tukey's HSD test showed that all means differed significantly from each other except for scraped and non-scraped old sediments. These differences were driven mainly by the large increase in chl *a* on scraped new sediments over the course of the experiment (Tukey HSD; Figure 4.9b).

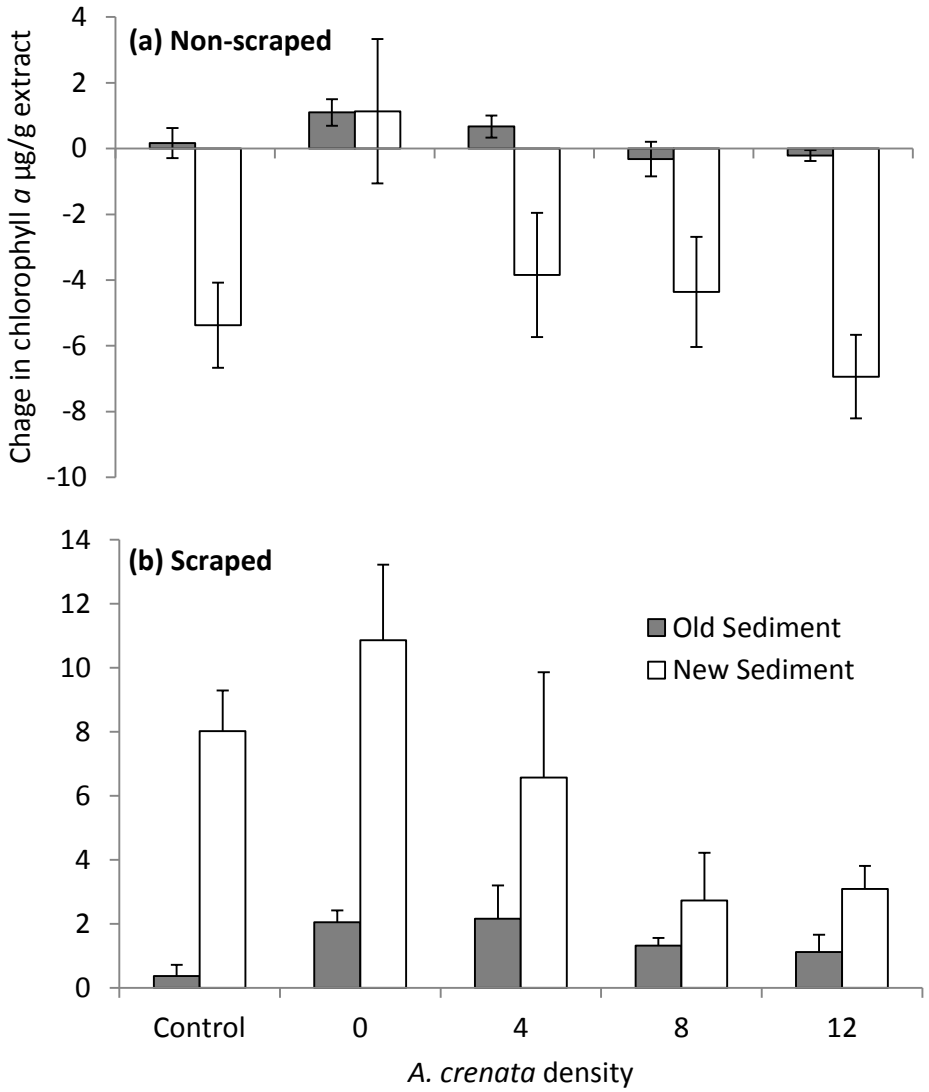

Figure 4.9: Mean (±SE) change in chl *a* concentration (µg/g extract) over 13 (old sediments) or 14 (old sediments) days in the *in situ* grazer cages, across different sediment types (new and old) and *Amphibola crenata* densities (0, 4, 8, and 12/625 cm^2) in the (a) non-scraped and (b) scraped cages. Note change in y axis scale between a and b.

There was also a significant interaction between sediment type and *A. crenata* density ($F_{4,220} = 3.63$, $p = 0.006$; Figure 4.9; Table 4.4a), which was driven primarily by a greater increase in chl *a* when the 0 density treatment was located in new sediments (Tukey HSD; Figure 4.9).

Table 4.4: General linear model results for the effect of different sediment types (new and old), scraping treatments (scraped and non-scraped) and *Amphibola crenata* densities (0, 4, 8 and 12/625 cm^2), over 13 (old sediments) or 14 (old sediments) days, on the mean (a) change in concentration of chlorophyll *a* µg/g extract, (b) ratio of phaeophytin *a*: chlorophyll *a* and (c) *Amphibola crenata* weight change.

(a) Mean change in chlorophyll *a* concentration (µg/g extract)

Source of Variation	df	SS	MS	F	p
Sediment type	1	7.20	7.20	0.32	0.57
Scraping treatment	**1**	**1901.34**	**1901.34**	**84.08**	**<0.0001**
***Amphibola* density**	**4**	**590.03**	**147.51**	**6.52**	**<0.0001**
Sed. type x Scr. treat.	**1**	**1216.08**	**1216.08**	**53.77**	**<0.0001**
Sed. type x *Amphibola* den.	**4**	**328.98**	**82.25**	**3.64**	**0.007**
Scr. treat. x *Amphibola* den.	4	37.79	9.45	0.42	0.80
Sed. type x *Amphibola* den. X Scr. treat.	4	91.18	22.79	1.01	0.40
Error	220	4975.23	22.61		

(b) Mean ratio of phaeophytin *a*: chlorophyll *a*

Source of Variation	df	SS	MS	F	p
Sediment type	1	0.00	0.002	0.51	0.48
Scraping treatment	**1**	**0.11**	**0.106**	**28.62**	**<0.0001**
Amphibola density	4	0.02	0.005	1.30	0.27
Sed. type x Scr. treat.	1	0.00	0.003	0.89	0.35
Sed. type x *Amphibola* den.	4	0.02	0.004	1.12	0.35
Scr. treat. x *Amphibola* den.	4	0.01	0.003	0.75	0.56
Sed. type x *Amphibola* den. X Scr. treat.	4	0.01	0.003	0.75	0.56
Error	219	0.81	0.004		

(c) Average *Amphibola crenata* weight change

Source of Variation	df	SS	MS	F	p
Sediment type	**1**	**0.19**	**0.19**	**5.99**	**0.019**
Scraping treatment	1	0.00	0.00	0.05	0.82
Amphibola density	2	0.02	0.01	0.26	0.78
Sed. type x Scr. treat.	1	0.09	0.09	3.01	0.09
Sed. type x *Amphibola* den.	2	0.00	0.00	0.05	0.95
Scr. treat. x *Amphibola* den.	2	0.02	0.01	0.26	0.77
Sed. type x *Amphibola* den. X Scr. treat.	2	0.05	0.03	0.81	0.45
Error	36	1.11	0.03		

In contrast to chl *a* the phaeophytin *a*: chlorophyll *a* ratio was not significantly affected by sediment type or invertebrate density ($p < 0.05$). Only the scraping treatment significantly altered the ratio, significantly decreasing it across old and new sediments ($F_{1,219} = 28.62$, $p < 0.0001$; Table 4.4b; Figure 4.10).

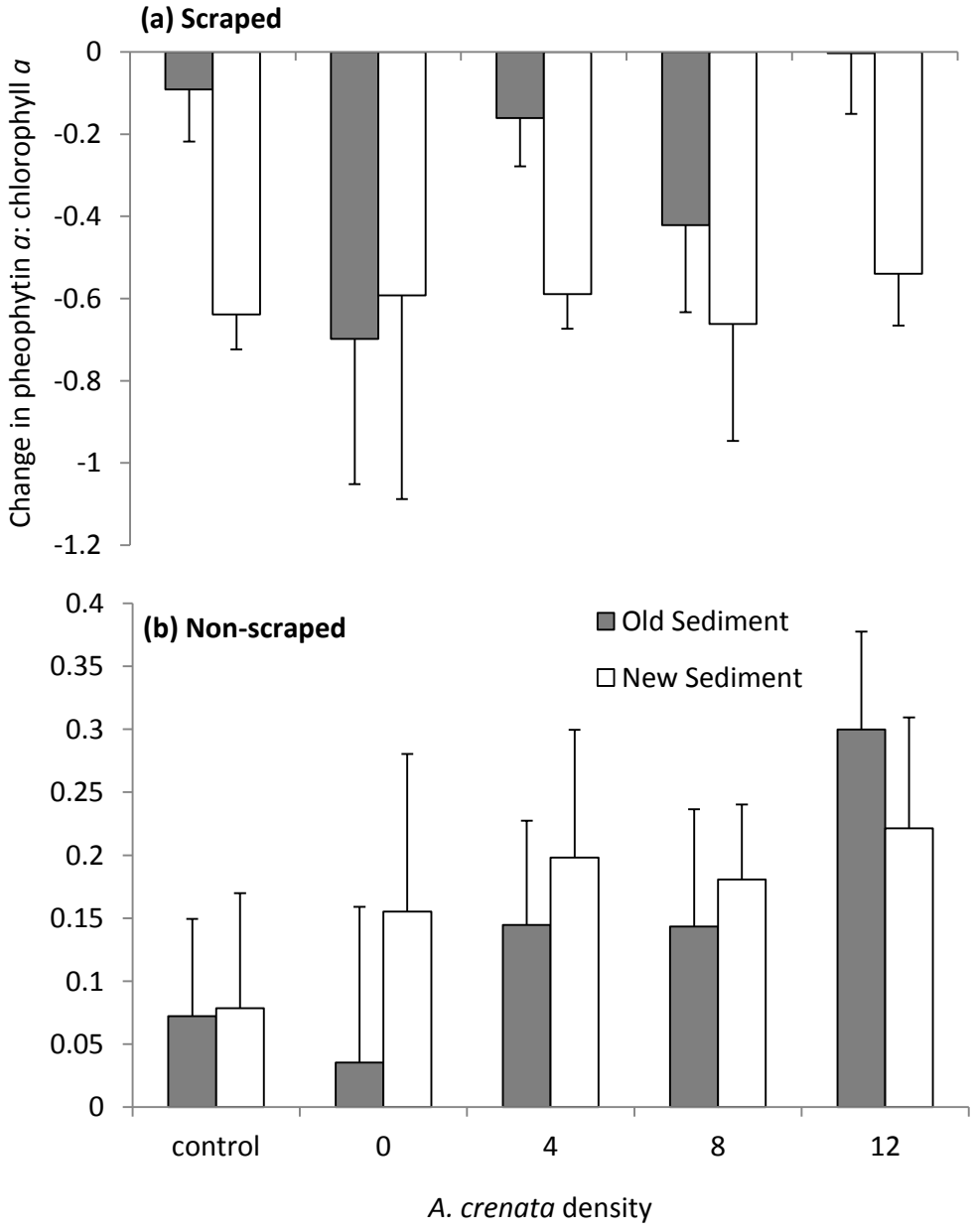

Figure 4.10: Mean (±SE) change in phaeophytin *a*: chlorophyll *a* ratio across *Amphibola* densities (0,4, 8, and 12/625 cm^2) and sediment types (new and old) in (a) scraped and (b) non-scraped cages. Note change in y axis scale between a and b.

All *A. crenata* remained within cages for the duration of the experiment, and no other animals were found in the new sediments. In the old sediments, a small number (1-2 individuals) of cockles (*Austrovenus stutchburyi*), *M. hirtipes* and juvenile *A. crenata* and were found in cages.

The linear regression between wet and dry weight of *A. crenata* collected prior to the experiment showed that the two are highly related (R^2 = 88%, $p < 0.001$; Figure 4.11). By the end of the experiments, the average dry weight of experimental *A. crenata* decreased significantly more in old sediments compared to new sediments ($F_{1,36} = 5.99$, $p = 0.019$; Table 4.4c; Figure 4.12).

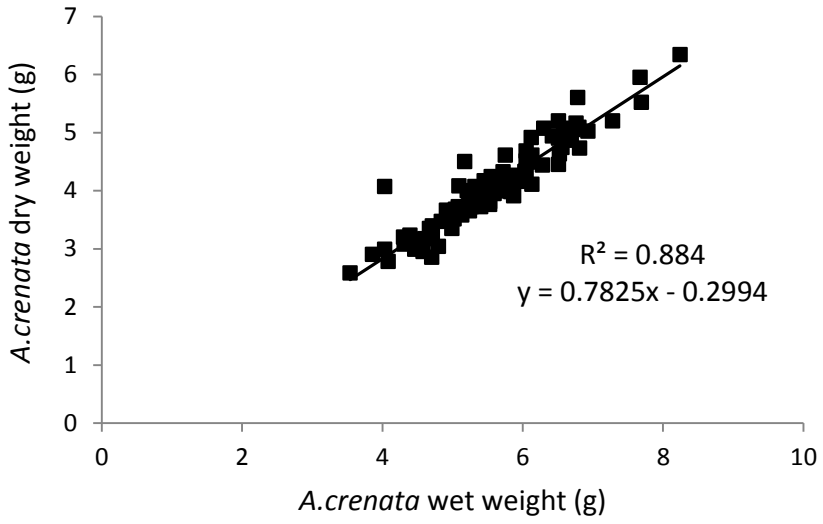

Figure 4.11: The relationship between *Amphibola crenata* dry weight (g) and wet weight (g), n =100.

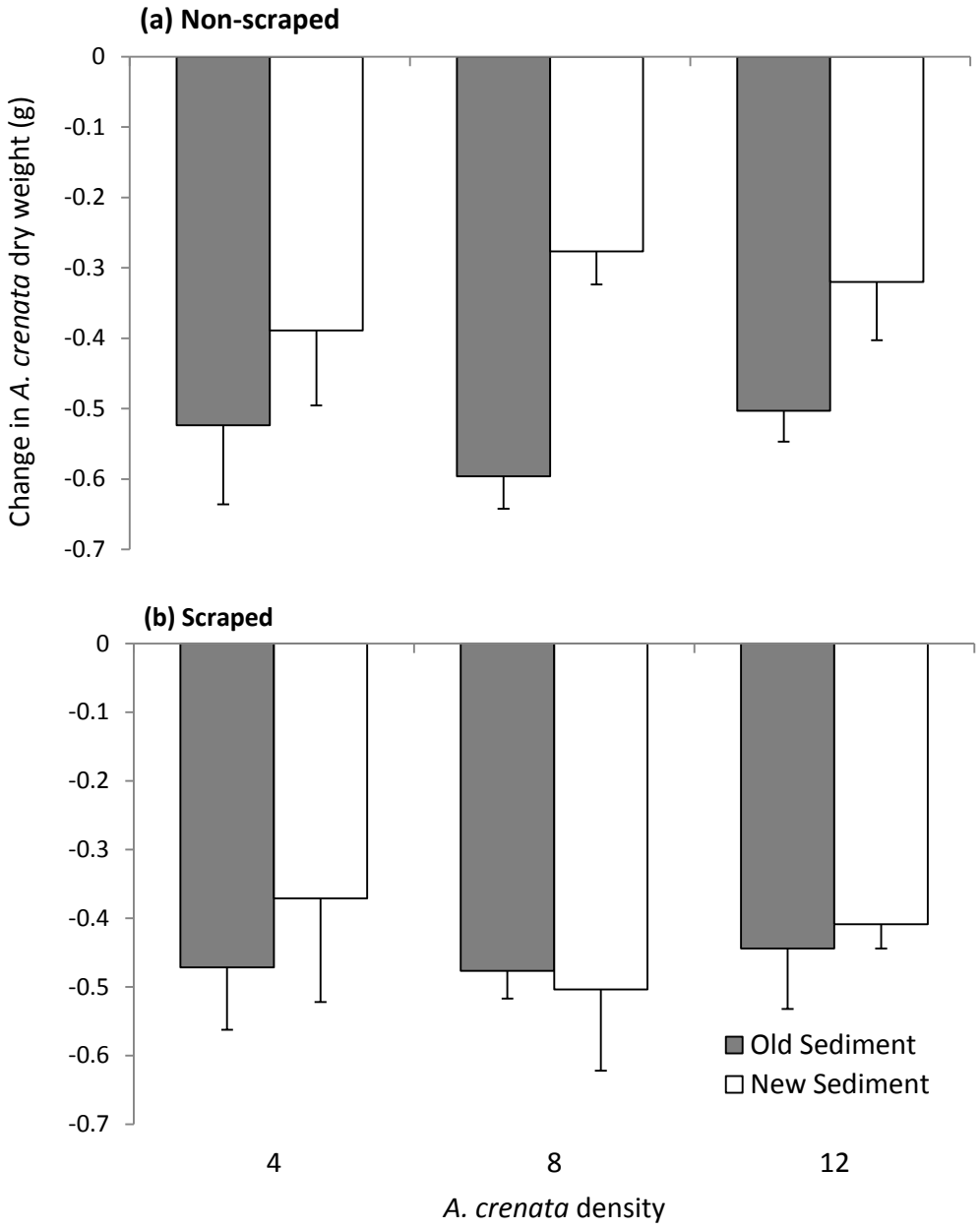

Figure 4.12: Mean (-SE) change in *Amphibola crenata* dry weight (g) in (a) non-scraped and (b) scraped *in situ* grazer cages, across different sediment types, and *Amphibola* densities over 13 (old sediments) and 14 (new sediments) days in the *in situ* grazer cages.

4.3.3 Laboratory examination of the indirect effects of invertebrate functional groups on benthic microalgal communities

Although no invertebrates completely escaped their enclosures, there were a few cases where crabs from the new sediments were found in the aquarium surrounding their core. In these cases, crabs were immediately placed back into their sediment cores. In old sediments, burrows could be seen in many invertebrate treatments. However, only *N. aestuariensis* constructed entirely subsurface burrows; *A. crenata* and *M. hirtipes* could frequently be seen with parts of their bodies exposed to the surface. Similarly, *N. aestuariensis* was the only invertebrate capable of burrowing in the coarse, new sediments. In these sediments, *A. crenata* and *M. hirtipes* created indentations in the sand, rather than burrows, and were frequently seen either sitting fully exposed on the sediment surface or trying to escape.

Chlorophyll *a* was significantly lower on new sediments than on old sediments ($F_{1,16} = 13.01$, $p = 0.002$; Figure 4.13a), consistent with the previous experiment. Chlorophyll *a* was significantly higher in all sediment types when polychaetes were present ($F_{3,16} = 7.39$, $p < 0.01$; Figure 4.13a) compared to control and crab treatments, but all other comparisons in the model were non-significant. Invertebrate type explained nearly twice the variation in chl *a* than sediment type did (40% and 23%, respectively).

The phaeophytin *a*: chlorophyll *a* ratio was significantly higher in old sediments than in new sediments ($F_{1, 16} = 4.96$, $p = 0.04$; Figure 4.13b).

At the end of the experiment, the cores were sieved for infauna. Only four of the 24 cores had infauna, all with a biomass less than 0.01 g and the only taxa were small (< 0.5 cm) polychaetes and *A. crenata*. Therefore, it is unlikely that the analyses for this experiment were affected by infauna within the sediment cores.

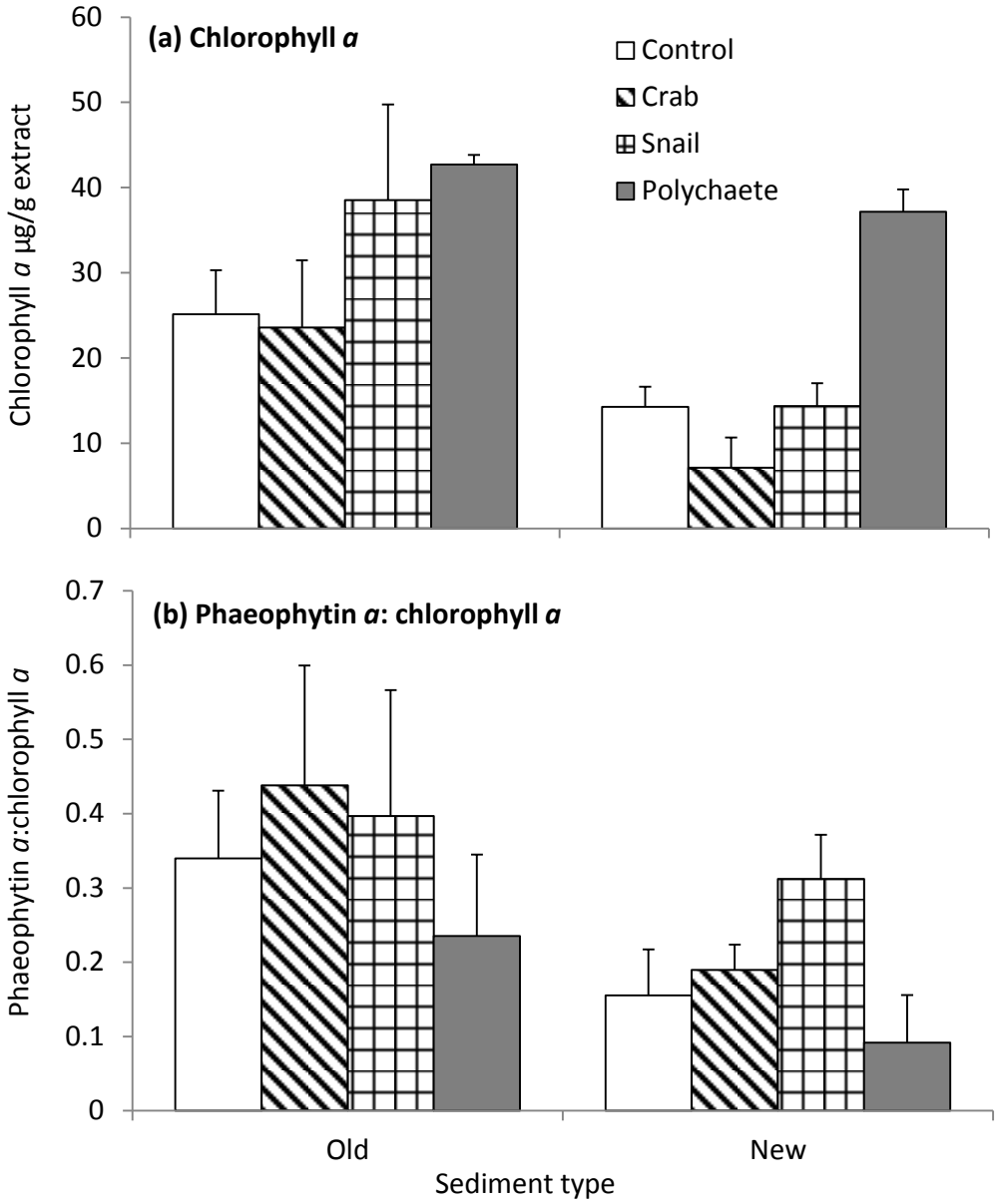

Figure 4.13: The mean (+SE) (a) concentration of chlorophyll *a* (µg/g extract) and (b) ratio of phaeophytin *a*: chlorophyll *a* in laboratory cores (50.3 cm^2) with different sediment (new and old) and invertebrate types (crab, snail, polychaete) present. Note change in y axis scale between a and b.

4.3.4 Macroalgal mats as indirect regulators of benthic microalgae on a mixed sediment substrate

Medium and thick mats of *Ulva* sp. significantly decreased the amount of chl *a* in the sediments, compared to the control treatments ($F_{3, 32} = 4.87$, $p < 0.01$; Figure 4.14). *Ulva* sp. thickness explained 82% of the variation in chla *a* concentration.

Although sediment underneath macroalgal mats appeared disturbed and darker than controls at the end of the experiment (Figure 4.15), it was shown that sediment grain size and organic content were not significantly affected by macroalgal treatment after seven days ($p > 0.05$ in all cases).

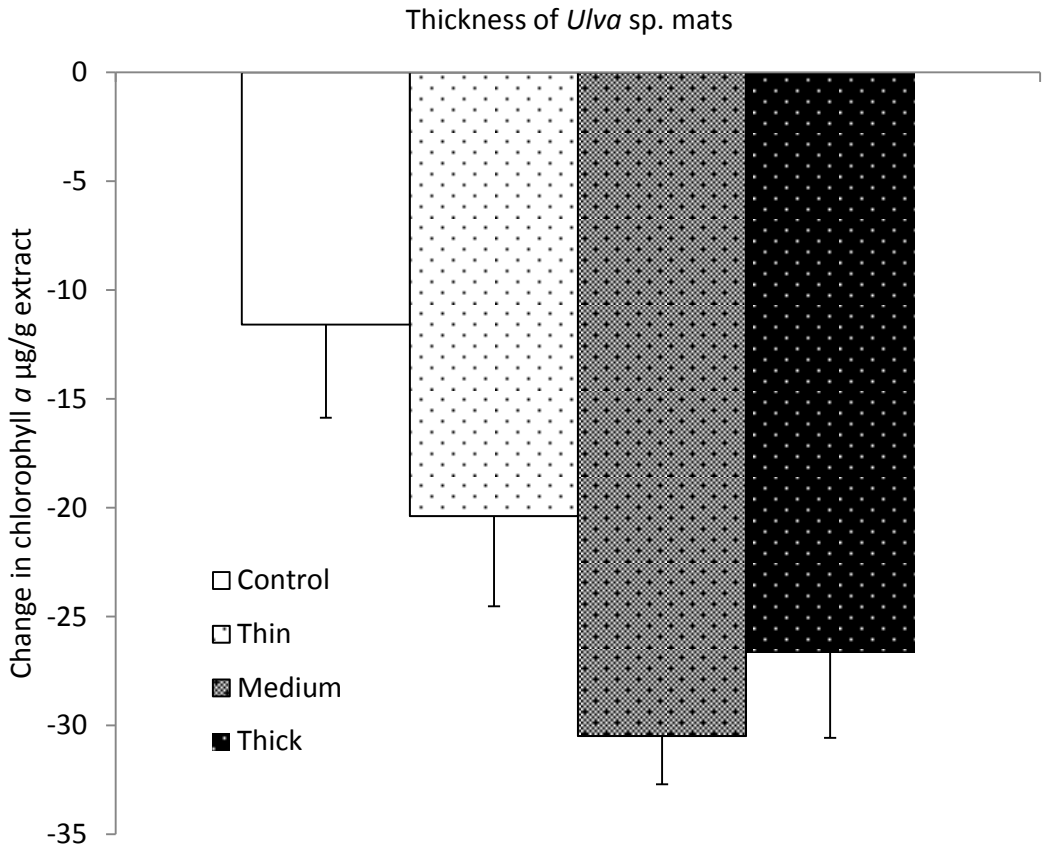

Figure 4.14: The effect of *Ulva* sp. thickness (control, thin, medium, and thick) on the mean (-SE) change in chlorophyll *a* concentration (µg/g extract) over 7 days.

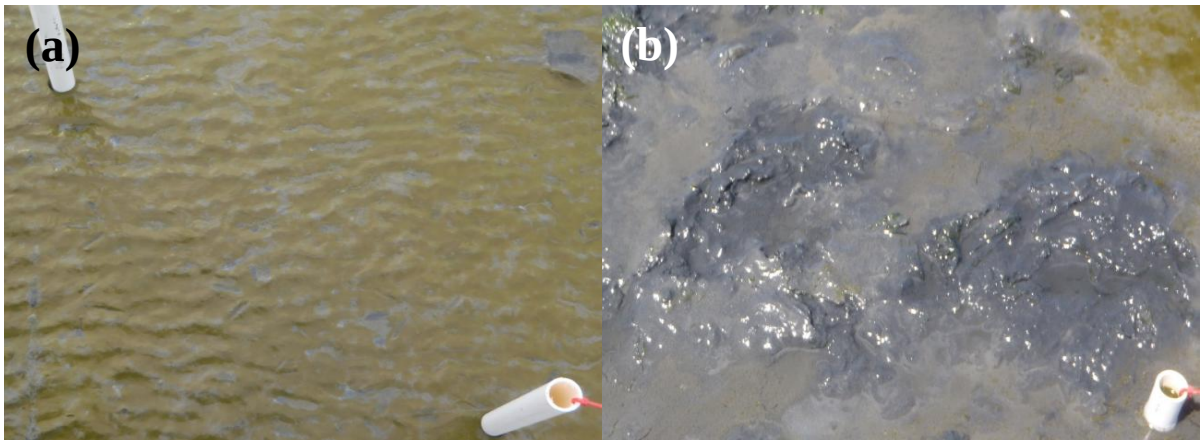

Figure 4.15: Change in the sediment surface underneath medium thickness (2 cm) *Ulva* sp. mats from (a) day 0 to (b) day 7. Photograph (a) depicts a relatively even surface with a thick layer of microalgae and (b) shows disturbed sediment with few visible microalgae left behind after the careful removal of an *Ulva* sp. mat.

There was a very strong positive relationship between chl *a* and light intensity across the thickness of *Ulva* sp. mats (R^2 = 0.89, $p < 0.001$; Figure 4.16), but this relationship is not definitive, and is unrefined, because the middle irradiance levels are not represented.

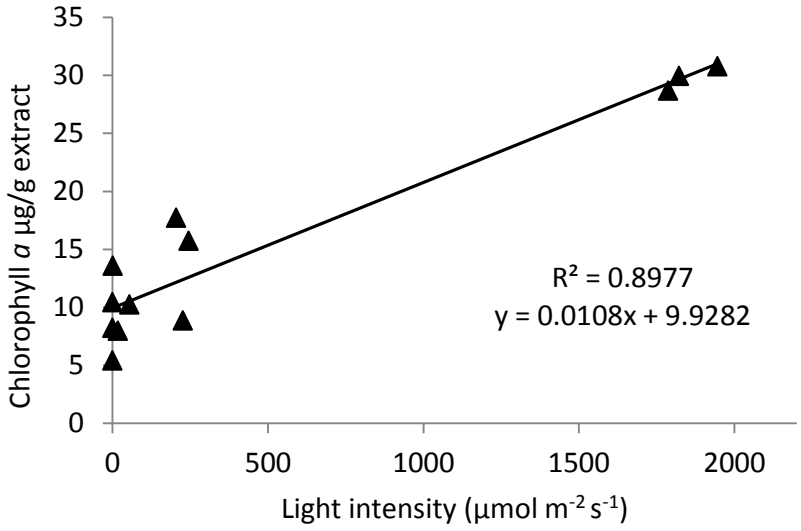

Figure 4.16: The relationship between the light intensity (µmol m^{-2} s^{-1}) underneath *Ulva* sp. mats and the mean chlorophyll *a* concentration (µg/g extract) after 7 days, n = 12.

4.4 DISCUSSION

Direct (invertebrate grazing) and indirect (bioturbation and the presence of competitors) interactions had a strong effect on the distribution and abundance of BMA, but these tended to be context and habitat specific. While some bioturbators can increase BMA biomass, grazing by invertebrates and the presence of dense and thick macroalgae can reduce BMA biomass. Sediment type alone had an effect on BMA biomass, and it was clear that, in some cases, functions and interactions involving new sediments were far different from those of old sediments. The nature and mechanisms of these interactions, and their implications for the recovery of the estuary, are discussed here.

4.4.1 Direct effects of macroinvertebrates on benthic microalgal communities

Scraping removed an average of 63% of BMA biomass in the new sediments but only 20% in old sediments. This difference between sediment types was surprising because it is generally considered that around 70% of benthic microalgae are located in the top 1 mm of sediment (Longphuirt et al. 2009) and so it was expected that nearly all algae would be removed by scraping off the top 1 cm. It appears, therefore, that the new sediments, at least at the experimental site, support a rich photosynthetic surface film of microalgae, while the old sediments do not. The surface film, found in the top few millimetres (Pinckney & Zingmark 1993) in the new sediments, would have been removed with scraping, accounting for the larger decrease in BMA biomass, compared to old sediments. Microalgae below 5 mm are not able to photosynthesise because of the severe reduction of light at these depths, so any algae found there are likely to be the result of physical mixing processes (Pinckney & Zingmark 1993). The homogenous vertical profile of BMA found in the old sediments is indicative of such processes (MacIntyre & Cullen 1995), and so a large decrease in BMA biomass would not be expected with scraping. Experimentally, however, the scraping of both sediment types effectively made the treatments equal in terms of BMA biomass at the start of the experiment, so that subsequent changes could be ascribed to how these sediments responded to the experimental treatments.

It was clear that grazing by *A. crenata* had a significant effect on BMA and that this varied between the laboratory and *in situ* experiments. At the Heathcote River Mouth (site of the *in situ* experiment), the effect of BMA varied between old and new sediments. Old sediments

had a very low chl *a* content at the end of the two week experiment and the density effect of *A. crenata* was less pronounced. For example, in the 0 density treatment, old sediments had less than one fifth of the chl *a* levels of the new sediments. In the new sediments, there was a stepwise reduction in mean chl *a* from snail densities of 0-8 but no further reduction in density 12. In cores from Humphreys Drive (used for the laboratory experiment), *A. crenata* had similar effects between both sediment types. It is difficult to determine the exact reasons for differences in density effects between the two experiments because there is a large disparity of studies comparing distributions of chl *a* between sediment types with low and high densities of infauna (Hagerthey et al. 2002). However, in the *in situ* experiment, the similarity in BMA biomass between 0 density treatments and controls, and the weight loss of *A. crenata* in the old sediments at the Heathcote River Mouth indicated that either there was little grazing on these sediments, or that food was of insufficient quantity or low quality.

This result was unexpected because it was originally hypothesised that *A. crenata* would not be able to graze on new sediments, because my distributional studies indicated that they seem to avoid new sediments. The greater decrease in chl *a* in non-scraped new sediments compared to non-scraped old sediments in my *in situ* experiment points to higher grazing activity by *A. crenata* on new sediments, which therefore appear to be a more suitable feeding substrate than the old sediments. Although an increase in chl *a* in the scraped version of the new sediments could point to less intense grazing pressure on new sediments, it is possible that food quality varied after scraping. This could be due to a change in the size or taxonomic composition of the recolonising BMA, compared to ambient populations. If microalgae are a certain size, they are able to avoid predation due to the morphological constraints of grazer mouthparts (Lubchenco & Gaines 1981, Steinman et al. 1987). Furthermore, some invertebrates are capable of preferentially grazing on certain species of BMA, so a change in BMA taxonomic composition could also be responsible for increases in BMA biomass on scraped new sediments (Buffan-Dubau et al. 1996, Hagerthey et al. 2002).

The greater densities of *A. crenata* in old sediments was also hypothesised to be due to an inadequate amount of microalgal food resources on new sediments compared to old sediments. Although BMA growth and recolonisation was higher in the old sediments at Humphreys Drive, this was not the case at the Heathcote River Mouth. At this site, BMA biomass increased in scraped new sediments, but not scraped old sediments, and did not increase in the absence of *A. crenata* in old sediments, further indicating that new sediments were a better substrate for BMA recolonisation and growth. Given the differences in physical

characteristics of the new and old sediments (Section 2.3.1), it was not surprising that sediment type was a driver of change in BMA biomass in these experiments and in the invertebrate functional group experiment. Potential reasons for these effects are summarised in Chapters 2 and 3, and include factors such as sediment grain size, stability, nutrient level and organic content. The inability of BMA to flourish, and of *A. crenata* to graze effectively, in old sediments at the Heathcote River Mouth was unexpected because *A. crenata* had thrived in these sediments for several years prior to the emergence of the new sediments (Owen 1992). Juniper (1987) showed that the amount of BMA in *A. crenata* diets can vary from site to site in the AHE, depending on the availability and primary productivity of the BMA. In some cases, 50% or more of the carbon requited for respiration came from non-microbial sources, and included non-living detrital carbon, dissolved organic carbon and meiofauna. Furthermore, Kanaya et al. (2008) showed that, in the vicinity of freshwater input the contribution of riverine-terrestrial materials to deposit feeder diets increased by up to 15%. It could be that, due to the very low biomass of BMA at the Heathcote River Mouth, *A. crenata* were not historically feeding on BMA in the old sediments, and relied on other food sources instead. With the appearance of new sediments, which are capable of supporting the growth of BMA, *A. crenata* may have only recently begun to incorporate BMA into their diet at this site.

Even though some gastropods, such as *Hydrobia*, can be size selective in ingesting particles, it has been shown that their densities and distribution are not correlated with particle size (Barnes & Greenwood 1978) and that it is likely that a suite of environmental factors, such as water movement and moisture retention, are responsible for invertebrate distribution (Barnes & Greenwood 1978). Huettel et al (1996) found that there was comparatively higher turbulence, desiccation and photoinhibition in convex sediment features, such as the new sediment mounds in my study, compared to concave sediment features. The distribution of *A. crenata* is likely to be partially accounted for by similar factors, but the observations that *A. crenata* had difficulties burrowing into the new sediments (Section 4.4.2) indicates that the ease of bioturbation may also be an important factor underlying *A. crenata* distribution. Erosion would be expected to be greater in new sediments, which are more coarse and less cohesive, making them more susceptible to resuspension (Needham et al. 2010) and, therefore, more difficult to construct stable burrows in.

Although it is possible that erosion accounted for some of the differences in BMA biomass in new sediments, these experiments indicate that *A. crenata* was capable of grazing on both

sediment types. In some cases, invertebrates can alter their feeding modes to exploit the food resources available (Biles et al. 2002, Needham et al. 2010), and while some estuarine species can change from deposit to filter feeding, other species that exhibit only one type of feeding mode may still exhibit a degree of flexibility to a change in environmental conditions (Biles et al. 2002). However, analyses of gut contents or faecal matter are needed to determine conclusively if *A. crenata* were grazing on both sediment types. There is also a possibility that the impact of other invertebrates, which were found in very small numbers in old but not new sediments, had at least a small influence on the results of the *in situ* experiment. The most abundant species, however, was the crab *M. hirtipes*, which was shown not to have a significant effect on BMA biomass in another experiment (Section 4.4.2).

4.4.1.1 The use of phaeophytin a as a marker of invertebrate grazing

The phaeophytin *a*: chlorophyll *a* ratio was only indicative of invertebrate grazing intensity in the *A. crenata* laboratory experiment, where the ratio increased with *A. crenata* presence and density. The ratios in the *in situ A. crenata* experiment appeared to mirror grazing activity in some cases, but overall, were not indicative of grazing pressure. Scraping of old and new sediments caused a decrease in the phaeophytin *a*: chlorophyll *a* ratio over two weeks, which would be expected because *A. crenata* may not have been grazing as efficiently on scraped sediments due to recolonisation by non-preferred BMA taxa or size (see above). However, since *A. crenata* grazing did occur in non-scraped new sediments, phaeophytin *a*: chlorophyll *a* should have increased in non-scraped sediments. Because there was no associated significant change in the ratio, it cannot be considered a useful marker of *A. crenata* grazing in the *in situ* experiment. Similarly, in the invertebrate functional group experiment (Section 4.2.3), the ratio did not mirror grazing activity in the sediments; the differences in chl *a* between *A. crenata*, *M. hirtipes*, and *N. aestuariensis* treatments indicated that differences in phaeophytin *a* should have been found, but they were not. Although reworking of the sediments by invertebrates could have stimulated the breakdown of phaeophytin *a*, causing it to degrade more rapidly into colourless residues that are undetectable via spectrophotometric methods (Ford & Honeywill 2002), this was not likely the case here. This is indicated by the control treatments, which were absent of fauna and did not differ from invertebrate treatments in their phaeophytin *a:* chlorophyll *a* ratios. Ford and Honeywill (2002) noted a complete absence of phaeophytin *a* in the faeces of grazers. Some researchers argue that it could be that the faecal pellets in their experiment had been reingested by invertebrates, resulting in complete degradation of phaeophytin *a* to colourless residues (Gieskes et al. 1991). However,

as control and invertebrate treatments did not differ in my experiment, this is unlikely. Although pigment degradation could also be used to explain the moderate relationship between chl *a* and phaeophytin *a* found in my study, this was probably not the case here (as above), and it is clear that a suite of variables may be capable of affecting phaeophytin *a* concentrations. I conclude, therefore, that it is unrealistic to estimate macroinvertebrate grazing intensity in the AHE using pigment analysis of phaeophytin *a*.

4.4.2 Indirect effects of invertebrate functional groups on benthic microalgal communities

In old and new sediments, BMA biomass was significantly increased in the presence of *N. aesturariensis,* compared to *M. hirtipes* and controls. Builders of extensive burrows, such as nereid polychaetes, have been shown to change the sediment environment through an extension of the sediment-water interface (Gerino et al. 2003). This increases the surface area for microorganism habitat, oxidative exchange, and translocation of particles (Gerino et al. 2003). Positive effects on BMA have been attributed to increased primary production, sediment metabolism and nutrient flux, as well as removal of toxic metabolites from the sediment (Tsuchiya & Kurihara 1979, Andersen & Kristensen 1988, Hansen & Kristensen 1998, McLenaghan et al. 2011). Tsuchiya and Kurihara (1979) demonstrated that the faeces of the polychaete *Neanthes japonica* (izuka) became food for diatoms, cyanobacteria and protozoans and that these microbes then increased in number. It is possible that this mechanism is partially responsible for the increase in BMA seen in my study, but it likely acted in combination with a variety of factors, caused by bioturbation, that influenced BMA. *Nicon aesturariensis* was able to construct burrows in both sediment types and, accordingly, they had similar effects on new and old sediments. Other studies have shown that invertebrates can alter their burrow morphology and feeding behaviour to help exploit the food resources that are available (Biles et al. 2002, Needham et al. 2010).

Because polychaetes intensively burrow and irrigate sediments, it was hypothesised that the effect of *N. aesturariensis* on BMA would be significantly greater when compared to *A. crenata,* a surface disturber, and *M. hirtipes*, a temporary burrow builder. However, neither of the latter two species exerted a significant effect on BMA biomass when compared to *N. aesturariensis*. Andersen and Kristensen (1988) found that the effects of a gastropod snail, *Hydrobia,* were less than that of a nereid polychaete because the shallow surface excursions

of *Hydrobia* did not stimulate microbial activity to the same extent as the deep burrows of the polychaete (Andersen & Kristensen 1988). A similar pattern was shown in my experiment, but the high variability between *A. crenata* treatments is likely a contributor to the lack of significance observed. It is also possible that one *A. crenata* was not sufficient to exert a significant effect on BMA, as seen in a previous study (Section 4.3.1). The lack of a significant effect of crabs on BMA biomass was in contrast to a study by Armitage and Fong (2006), where the burrow-excavating crab, *Pachygrapsus crassipes*, decreased chl *a*, probably through a mixture of grazing and the physical disturbance of microalgal mats from bioturbation. The inability of some experimental cores to confine *M. hirtipes* could partially explain why no effect on BMA biomass was seen in my study. The crabs seemed to avoid both sediment types and were sometimes found in the surrounding aquaria, which indicated that they were able to escape through the mesh top and swim out of the core on the high tide cycle. Furthermore, *M. hirtipes* burrows were not seen in the new sediment and crabs were often seen sitting on top of the sediment, possibly due to the unstable nature of the substrate. Partial burrows were present in old sediments, but the ability of crabs to escape may have prevented the construction of more elaborate burrows, as well as diminishing the potential for crabs to have a significant impact on BMA biomass. Needham et al. (2010) also found differences in the burrows of crabs between sediment types. As silt-clay content of the sediment increased, the burrows of *Austrohelice crassa* became deeper, more permanent and more stable than those in fine, clean sand (Needham et al. 2010). Much of these differences were attributed to the more cohesive nature of small sediments. Similar to my experiment, burrows in sandier sediments usually collapsed, which would undoubtedly have altered the way *A. crassa* interacted with the sediments.

4.4.3 Macroalgal mats as indirect regulators of benthic microalgae on a mixed sediment substrate

Medium and thick mats of *Ulva* sp. decreased BMA biomass on surfaces composed of both new and old sediments in the AHE. A large amount of the variation in chl *a* concentration was explained by the presence of *Ulva* sp. (82%), and it was likely that the mechanism for this was a reduction in light underneath the mats. There appeared to be a threshold effect of light on BMA biomass; medium and thick macroalgal mats blocked out 99 and 100% of light, respectively and significant decreases in BMA biomass were not seen in other treatments,

where light intensities were within the known saturation values for intertidal diatoms (Blanchard & Montagna 1992, Pinckney & Zingmark 1993, MacIntyre & Cullen 1995). Lower amounts of light reaching the sediment would have caused a decrease in BMA photosynthesis and biomass (Corzo et al. 2009, Garcia-Robledo et al. 2012). These results are in accordance with Sundback et al. (1996), who found that when green algal mats reduced light intensity by more than 99% there was a resulting change in microalgal biomass. The lack of significant effect of 100% cover treatments on BMA biomass was unexpected because 100% of light was blocked out in this treatment. Physical mixing processes and horizontal migration by BMA may have contributed to the chl *a* found in these sediments.

Ulva can absorb nutrients directly through its surface, and can grow rapidly, so competition for nutrients is one way in which this macroalgal species can alter BMA biomass (Owen 1992, Corzo et al. 2009). Oxygen under macroalgal mats can be also be decreased, due to reduced water velocity, and the aerobic respiration of the macroalgae in the dark (Corzo et al. 2009). The decrease in oxygen favours an increase in anaerobic processes (Bressington 2003), which can inhibit microalgal growth (Graham & Wilcox 2000). Anoxia may also explain the consistent dark appearance of the sediments after macroalgal mat removal. The appearance of dark sediments were also noted by Sundback et al. (1996) in a similar experiment, but after observing that sediments became the same colour as the control sediments after being placed in the sun, it was concluded that the dark appearance could be attributed to the vertical migration of diatoms within the sediment (Sundback et al. 1996). Downward migration of BMA in the sediments may also account for the dark surface of the sediment in my study, but anoxia cannot be ruled out.

This study is one of the few studies examining the competition between different types of algae (Sundback et al. 1996) and indicated that while large amounts of *Ulva* sp. can decrease BMA biomass, microalgae do not appear to be limited in abundance by overlying green macroalgae when they are thin and sparse. It must be remembered that such dense mats are frequent occurrences in parts of the estuary, particularly during the summer months. Therefore, it is likely that at least some of the variation in BMA among sites, particularly near shore where drift macroalgae can accumulate, is due to interactions with macroalgae.

4.4.4 Summary

The importance of biotic interactions in regulating BMA biomass and distribution, and the changes in functioning after disturbance and during recovery, are not well understood (Davis & Lee 1983, Lohrer et al. 2010) and so this study makes an important contribution in understanding some underlying mechanisms. Taken together, the results here show that the trajectories of mixing, change and recovery of new and old sediments could materially affect functional interactions of invertebrates in the estuary, particularly with reference to production and use of BMA. However, it appears that the strength of this effect on BMA biomass is site-specific and that, at least in some cases, the benthic species are well adapted for a broad range of environmental change. To better predict the patterns of recovery from the recent disturbances caused by the earthquakes, other factors, such as biogeochemical influences, need to be more thoroughly understood. As it has been shown that the influence of invertebrate effects on BMA can be altered by their interaction with bottom-up forces (Cranford 1988, Hagerthey et al. 2002, Hillebrand & Kahlert 2002), another important step is to evaluate all of these ecosystem components together.

Chapter 5

Synthesis

5.1 INTRODUCTION

This study tested and clarified the effects of earthquake-driven environmental change on benthic microalgae (BMA) in a highly eutrophic estuary. Specifically, grain size, organic content, ammonium concentrations and biological interactions were all impacted and affected by the earthquakes, and these factors directly and indirectly influenced BMA distribution, biomass, production and community structure in the Avon-Heathcote Estuary (AHE). Sediments and nutrients are important in controlling the distribution and abundance of BMA in estuaries because of their direct effects on the immediate physical environment and habitat, and their associated indirect effects on the biological interactions of invertebrates and macroalgae with BMA (Thrush 1991, Underwood & Provot 2000, Bressington 2003, Cartaxana et al. 2006, Montserrat et al. 2008, Brito et al. 2009). This study has demonstrated that BMA change can be positively or negatively driven by such direct and indirect effects. This is important because, as large contributors to primary production and the main drivers in nutrient cycling, BMA enhance the resilience of estuaries to disturbance, and contribute to the rapid recovery of eutrophic estuaries (Larson & Sundback 2012).

Rates and patterns of ecosystem recovery can be context-dependent or site-specific due to interactions with the physical characteristics of an area (Norkko et al. 2010), so it was important to analyse how BMA had been altered by the earthquakes at different locations in the AHE. The sites near the mouth of the estuary differ in their physical and biological characteristics, with coarser sediment grain size and less eutrophication than those sites near the head of the estuary (Knox and Kilner 1973, Bolton-Ritchie 2011b). This is due to differences in hydrodynamic patterns and the proximity of the sites to sources of large amounts of organic matter and nutrient stores in the sediments (Knox & Kilner 1973). Although different experiments were used to study the sites in some cases, it was still

possible to compare general patterns and processes that occurred after the earthquakes, and to gauge the trajectory of BMA recovery in the estuary.

5.2 CONSEQUENCES OF EARTHQUAKE-DRIVEN CHANGE ON BENTHIC MICROALGAL DISTRIBUTION AND ABUNDANCE

The emergence of new sediments and the addition of wastewater to the estuary during the Canterbury earthquakes of 2010 and 2011 altered the estuarine ecosystem, starting at the base of the food chain with the BMA. In this study, new sediments covered up to 65% of the estuary floor and generally altered the physical habitat by increasing the mean grain size, and decreasing the organic content, ammonium concentration, stability, macroalgal cover and invertebrate density in and on the substrate. Invertebrate and macroalgae densities were reduced on new sediments because new sediments were less stable, due to their coarser grain size, and not conducive to macroalgal attachment or invertebrate burrowing activities. The lower amounts of organic matter and ammonium in the new sediments was the result of their fairly recent emergence into the estuary. However, levels of these are still considered high and reflect the quick accumulation of the large amounts of sewage and nutrients that were released into the estuary after the earthquakes. Together, the above factors resulted in changes in BMA distribution over six months, and a decrease in biomass by around 12-93% (depending on site and time), indicating that new sediments were less favourable for BMA growth and recolonisation. This could be due to the interactions between BMA and the suite of variables that were altered by the new sediments. For example, BMA showed positive relationships with the percentage of fine sediment, organic content and some invertebrate bioturbators (polychaetes), and negative relationships with macroalgal cover, infaunal biomass, ammonium concentrations at the sediment surface and densities of the grazing gastropod *Amphibola crenata*. Experimentally increased sediment and water column nutrient levels did not alter BMA biomass, highlighting that BMA are no longer limited by nutrients in the highly eutrophic AHE and that they are more strongly limited by other factors, such as grazing and sediment type.

It is clear that the strength and direction of the effect of the new sediments on BMA biomass is site-specific and that the benthic community is capable of adapting to a range of earthquake-induced environmental changes. Microalgal biomass was greatly decreased on new sediments, compared to old sediments, at Humphreys Drive (a highly eutrophic site), but

not at Plover Street (one of the least eutrophic sites). New sediments were less favourable to BMA growth due to larger grain sizes, and site differences were the result of differences in environmental gradients such as ambient grain size, level of eutrophication and hydrodynamic stress. Humphreys Drive is more favourable for BMA growth in general because the small sized sediments are more cohesive than those near the mouth of the estuary, it is in close proximity to the old outfall of the wastewater treatment plant, and the hydrodynamic stress is low. There were also site-specific differences in grazing and it was apparent that, in some cases, functions and interactions involving new sediments were different from those of old sediments. For example, at the Heathcote River Mouth grazing by *A. crenata* was more intense on new sediments compared to old sediments. At this site, the new sediments were a better substrate for BMA growth and this was indicated by the larger BMA biomass and greater recolonisation capabilities after removal of ambient BMA mats. This was unexpected because monthly monitoring indicated that BMA biomass tended to be higher in old sediments, which contained higher amounts of organic content and smaller grain sizes. In terms of the adaptability of the benthic community to earthquake-induced environmental change, it is notable that increased sediment and water column nutrient loads did not significantly affect BMA, invertebrate and macroalgal density, and that BMA were capable of growing on new sediments, particularly at the Heathcote River Mouth.

It is difficult to compare these results to those of other studies, as there has been no previous research on the effects of earthquakes on BMA in an estuarine environment. The most similar studies to mine involve the comparison of BMA distribution, abundance, or taxonomic composition between a variety of grazing intensities, macroalgal covers, nutrient levels, organic loads or eutrophication gradients (Steinman et al. 1987, Sundback et al. 1996, Underwood et al. 1998, Underwood & Provot 2000, Piehler et al. 2010, Weerman et al. 2011). However, none of these studies examine how these factors interact with different sediment types to alter BMA communities, and this is problematic because it has been shown that the interaction of bottom-up, top-down and disturbance events act to regulate BMA (Hagerthey et al. 2002). The experiments in my study highlight the importance of taking an ecosystem approach to the understanding of how cataclysmic disturbances can alter estuarine BMA distribution and abundance; a variety of direct and indirect influences on BMA were capable of being altered by sediment type, and there was also a strong effect of site within the AHE. More eutrophic sites tended to show greater differences in the characteristics (grain size, organic content) of new and old sediments, as well as larger reductions in BMA biomass

after the emergence of the new sediments. It is, therefore, important to realise that earthquake-induced effects of BMA in the AHE, although substantial in many cases, can be both extensive and context-specific.

5.3 SPATIAL AND TEMPORAL SCALES IN THE RECOVERY OF BENTHIC MICROALGAE AND THE AVON-HEATHCOTE ESTUARY

The AHE showed initial signs of recovery from eutrophication after the wastewater diversion in 2010 (Zeldis et al. 2011), but the series of earthquakes that began in September 2010 had lasting effects on the BMA, altering the recovery trajectory of the estuary. There is a large load of legacy nutrients stored in the new and old sediments, creating a diffusion gradient towards the water column. However, the ability of BMA to use the legacy nutrients from within both old and new sediment types will prevent these nutrients from being reintroduced into the water column, which would otherwise cause algal blooms and delay the recovery of the estuary (Hu et al. 2001). This indicates that BMA play an important role in ecosystem function and are crucial in helping the estuary return to a non-eutrophic state (Little 2000, Piehler et al. 2010). The decreases in their biomass in the new sediments could lead to interferences in recovery from eutrophication and a loss of resilience to future disturbance events (Graham & Wilcox 2000, Lohrer et al. 2010, Piehler et al. 2010, Larson & Sundback 2012). Unfortunately, recovery of BMA in the new sediments appeared to be slow after the earthquakes. In some cases, nearly a year after the February and June 2011 earthquakes, BMA biomass had not equilibrated in the new and old sediments. It is expected that, over time, the BMA community will recover to its pre-earthquake state, but that the rate of this will be strongly influenced by site and small scale heterogeneity in environmental gradients, as discussed above.

Although the lower BMA biomass in the new sediments could decrease overall rates of nutrient cycling in the AHE, recovery of the estuary may not be compromised by the emergence of the new sediments, which were beneficial to the functioning of the estuary in some cases. New sediments tended to have lower organic content than the old sediments at the most eutrophic sites. Through deposition of organic matter, ammonium can be stored in the sediments for long periods of time and eventually released through breakdown by microbial processes. Combined with lower ammonium concentrations, the decreases in organic matter in new sediments could act to speed the recovery of the estuary by decreasing

the amount of legacy nutrients in the sediments. Furthermore, taxonomic composition and primary productivity were not significantly different between new and old sediments, indicating that BMA are capable of cycling nutrients in both sediment types. At the Heathcote River Mouth the new sediments actually acted to increase functioning and resilience to disturbance because they provided a substrate for BMA growth and invertebrate grazing. Due to a greater capability for grazing on BMA, this site may now be able to support a higher density of *A. crenata* and a more diverse assemblage of invertebrate species, increasing ecosystem functioning in the future. Therefore, in terms of estuary wide recovery, the most detrimental effect of the earthquakes was most likely the addition of large amounts of organic matter and nutrients, which increased nutrient stores in the sediments, rather than the emergence of new sediments.

This study did not span sufficient time to determine conclusively the length of time necessary for BMA and estuarine recovery from disturbance and eutrophication, but it is likely that it will proceed at different rates at different sites. At Plover Street, the least eutrophic site, new sediments did not differ from old sediments in physical characteristics, and BMA biomass was similar across both sediment types. Recovery of BMA at this site, if not already complete, will be quick. Conversely, at the most eutrophic site (Humphreys Drive), new sediments had a much reduced BMA biomass and larger grain sizes than the old sediments. Ammonium diffusion rates from new sediments were slow and organic breakdown to ammonium happened as fast as ammonium consumption by BMA. Therefore, recovery at eutrophic sites will be prolonged due to the larger amounts of nutrients and the more anoxic nature of the old sediments compared to the new sediments. Nonetheless, it is clear that the new sediments are similar to those found in less eutrophic areas and these sediments will, in some cases, help the estuary recover from eutrophication by burying the more organically enriched old sediments. Problems may arise if the new sediments, which are less favourable for BMA growth due to grain size, continue to be distributed over old sediments, where they will become highly enriched with organic material. This would be most detrimental at the highly eutrophic sites and in these cases, recovery could take years. Recovery will be prolonged further if major earthquakes and the associated emergence of liquefied sediments continue. With a disparity of research on BMA succession, more data needs to be collected on larger temporal and spatial scales, and more experiments need to be done on the factors that regulate such processes. The trajectories of mixing of new and old sediments also need to be monitored further to determine the future direction and rates of recovery.

5.4 SUMMARY

In summary, this study demonstrated the effects of earthquakes in structuring BMA communities and ecosystem recovery. No other studies have accomplished this to date. These data contribute to growing evidence that habitat modification and direct and indirect environmental influences can have significant effects of the recovery, distribution and abundance of BMA in estuaries, and that these effects are complex and altered by a variety of environmental gradients (Davis & Lee 1983, Meadows & Tufail 1986, Underwood 1997, Watermann et al. 1999, Hillebrand & Sommer 2000, Cartaxana et al. 2006, Janousek et al. 2007, Wardle 2009, Grinham et al. 2011, Larson & Sundback 2012). It is important to realise that various processes, such as nutrient flux and grazing, are responsible for regulating and maintaining BMA communities and consequently, the health of the estuary. It is still not fully understood how these processes and the complex interactions between flora, fauna and sediment biogeochemical factors have been altered following the earthquakes. Therefore, the trajectory of estuarine recovery is somewhat unclear and other factors, such as those listed above, will need to be examined and monitored to determine the direction and state of the recovery. This calls for further research on how basic processes and feedbacks have been altered by the earthquakes, and the use of a holistic approach to understanding the effects of disturbances. An accurate understanding of such things represents a significant step towards improving our ability to manage the AHE and to be able to evaluate reliably the effects of the wastewater diversion.

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APPENDIX 1

A review of frequently used methodology and equipment

1. SEDIMENT ANALYSIS

Total organic content

Most sediments contain widely disseminated decomposed products of tissues and other organic material (Gross 1971). In the Avon-Heathcote Estuary (AHE) the bulk of this material is derived from phytal material, but decaying tissue, faeces and excretions from living animals also contribute (Knox & Kilner 1973). The large supply of phytal material comes from two sources: autochthonous sources (submerged vegetation, macroalgae and benthic microalgae from within the estuary) and allochthonous sources (river borne detritus, effluents and windblown terrigenous material from outside the estuary; Knox & Kilner 1973). The total organic content can provide useful information about the sediment environment, such as the concentration of dissolved oxygen, productivity in the bottom waters, and the strength of bottom currents (Gross 1971).

Quantifying the amount of organic matter in sediments is of importance because organic detritus provides an important food source for many estuarine organisms (Pomeroy 1959). Separation of organic matter from sediments cannot be accomplished by physical means (Gross 1971), and so other types of analyses are required to gain information about the amount of total organic content. These techniques usually involve destruction of the organic material in sediments by oxidation, and quantifying its total by comparing final and initial weights of the sediment sample (Gross 1971). A relatively simple and efficient method for this is accomplished by high temperature ignition loss, as long as the sediments do not contain large amounts of clay, which decompose at high temperatures (Gross 1971). High temperature ignition loss involves the use of minimal equipment and is, therefore, useful in situations with little time and few resources (Gross 1971). The sediment profiles from the AHE in 2011 indicated that there was less than 5% clay present (Bolton-Ritchie 2011a), so

any loss to ignition would be minimal. Therefore, organic matter in my studies was determined as loss on ignition, in a modified version of the method outlined by the American Public Health Association (APHA 1965).

Large debris was removed from each sediment sample and the entire sample was mixed using a metal spoon. Sediment (around 15 g) was removed from the sample to fill a pre-weighed porcelain crucible. This was then ground using a metal spoon, placed in the crucible and dried at 50°C until it reached a constant weight (about two days). The crucible was weighed to obtain the pre-ash weight of the sediment, transferred into a muffle oven and heated for five hours at 550°C. It was then removed from the oven and allowed to cool partially in a desiccator before being reweighed to obtain the post-ash weight. The percentage of total organic content was calculated as the:

(pre-ash weight - post-ash weight)/pre-ash weight x 100

Grain size

Analysis of sediment grain size is important in the evaluation of intertidal mud flats. The spatial variation in sediment size can provide information to help determine biological patterns in the environment, and temporal variation is indicative of events such as erosion or accretion (Buchanan 1984). The physical characteristics of sediments also have a profound effect on the chemical environment, and the interaction of these physical and chemical properties can alter the structure of biological communities (Buchanan 1984).

In my studies, sediment grain size was analysed by wet sieving, in a modified version of Ingram's method (1971), to identify the percentage of very coarse sand, coarse sand, medium sand, fine sand, very fine sand and silt-clay (< 63 µm). Sediment remaining in the sample (around 40 g) after the removal of sediment for organic matter analysis was placed into a container with 200 ml of water and homogenised using a metal spoon. The entire sample was placed on top of a tower of sieves that were arranged, from top to bottom, in order of descending mesh size (1000 µm, 500 µm, 250 µm, 125 µm, and 63 µm). The mesh size of the sieves was chosen using the Wentworth grade classification (Wentworth 1922; see Table 1). A two litre white bucket was placed underneath the stack of sieves to catch the sediment size fraction that passed through the smallest sieve (< 63 µm).

Table 1: Wentworth (1922) grade limits for sieve mesh sizes

Size Terms	Grade Limits
Boulder	> 256 mm
Cobble	256-64mm
Pebble	64-4mm
Granule	4-2mm
Very coarse sand	2000-1000 µm
Coarse sand	1000-500 µm
Medium sand	500-250 µm
Fine sand	250-125 µm
Very fine sand	125-63 µm
Silt	63-4 µm
Clay	< 4 µm

Distilled water was used to wash the sediments through each sieve, until the water running through each sieve was clear, indicating that sediment was no longer passing through. The sediment retained on each sieve was washed into a pre-weighed aluminum tray and put into an oven (50°C) until it reached a constant weight (1-3 days). The sediment and water remaining in the white bucket were put aside and not disturbed until all of the sediment had settled out of the water. The water was carefully decanted off the sediment and the sediment was transferred into the oven, as above. Once the sediment in the oven had dried, it was reweighed and the results for each sediment size were expressed as a percentage of the total dry mass of the sample.

2. EXAMINATION OF BENTHIC MICROALGAE

Using chlorophyll *a* as a proxy of benthic microalgal biomass

Pigments are widely used as biogenic markers for plant derived organic material (Ford et al. 1999, Ford & Honeywill 2002, Cartaxana et al. 2003, Thrush et al. 2009, Wardle 2009) and provide useful information about oceanic plant communities (Buffan-Dubau & Carman 2000). The widely examined pigment chlorophyll *a* (chl *a*) is the main photosynthetic pigment in nearly all oxygenic photosynthetic organisms and corresponds to living phytal material (Garrigue 1998, Ritchie 2006, Ritchie 2008). It can be used as a basis on which to calculate the productivity and metabolically active biomass of autotrophic organisms in aquatic systems, such as benthic microalgae (Revsbech 1989, Christensen et al. 1990, Ford &

Honeywill 2002, Ritchie 2006, Ritchie 2008). When using chl *a* concentration as a proxy of BMA biomass in sediments, it is important to sample only the top few millimetres of sediment, where photosynthesis takes place, as this is the most biologically active layer and where the majority of microalgae reside (Palmer & Round 1965, Longphuirt et al. 2009). Sampling the top layers with an accuracy of a millimetre without compacting the substrate can be very difficult, but "contact cores" (designed by Honeywill & Hagerthey and first cited in Ford & Honeywill 2002; see Figure 1) provide a reliable technique.

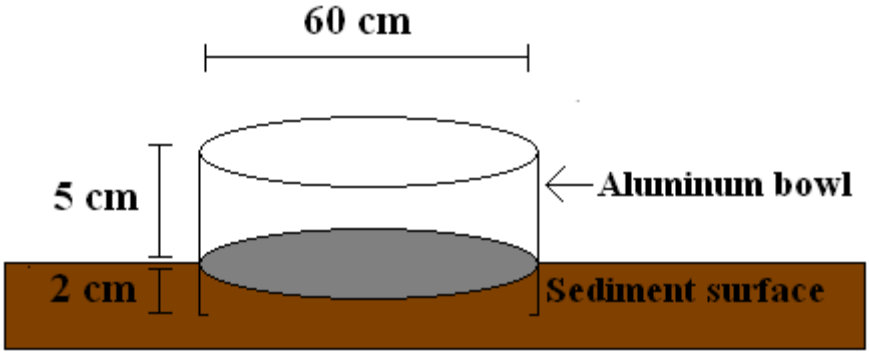

Figure 1: Diagram showing a contact core positioned so that the bottom of the aluminum bowl is flush with the sediment surface

A contact core is a simple bowl-shaped aluminium sampling device with a 2 mm high cavity on its bottom side (Figure 1). When using a contact core, it was first carefully pushed into the sediment until the bottom of the metal bowl was flush with the surface of the sediment. Liquid nitrogen was poured into the top of the bowl and allowed to evaporate until at least 2 mm of sediment beneath the contact core was frozen (Ford & Honeywill 2002). Freezing time was assessed for each sample and varied according to weather conditions and sediment water content, but was generally around 30 seconds. After the sediment was frozen, the contact core was removed (including the frozen sediment) and a sharp, flat knife was used to scrape the frozen sediment until it was flush with the contact core (Ford & Honeywill 2002). A flat frozen disc of sediment resulted, which was then removed and covered with labelled tin foil. The frozen sediment was immediately placed into liquid nitrogen until it could be stored in a -80°C freezer to avoid pigment breakdown (Ford & Honeywill 2002).

Before chl *a* was extracted, contact core samples were freeze dried until all of the moisture was removed (24 h), because Buffan-Dubau and Carman (2000) found that pigment

recovery was nearly three times higher in sediments that were freeze dried, compared to those that were not. Extraction methods were modified versions of those presented by Sartory and Grobbelaar (1984). Two grams of sediment from each sample were weighed out, placed in a falcon tube with 10 ml of 70% ethanol and shaken for ten seconds. Although a variety of solvents can be used to extract chl *a* (Sartory & Grobbelaar 1984), ethanol was chosen because it is considered to be the most safe, practical and economically feasible solvent (Sartory & Grobbelaar 1984, Ritchie 2006, Ritchie 2008). After the samples were shaken, they were immersed in a hot water bath (78°C) for six minutes, shaken for ten seconds to homogenise the sample and placed in the dark for twenty hours in a refrigerator (Sartory & Grobbelaar 1984). The samples were then shaken for ten seconds and allowed to sit for three hours so the sediment could settle out. The top 5 ml of each sample was pipetted into a glass tube and its absorbance was measured at 665 and 750 nm using a spectrophotometer (Shimadzu UV-1800 containing a sipper unit that removes 2 ml of sample per reading). Three drops of 1 M HCL were added to the glass tube, which was shaken gently and allowed to sit for three minutes before another reading was taken. It has been shown that phaeophytin *a*, a degradation product of chl *a*, can constitute a significant fraction of the total green pigment present in oceanic samples, and can introduce error into the chl *a* measurements because it absorbs light in a similar part of the spectrum as chl *a* (Parsons & Strickland 1963, Lorenzen 1967). Therefore, acidification of the sample is used to distinguish between chl *a* and phaeophytin *a* concentrations (Lorenzen 1967). Acidification degrades the chl *a* but not phaeophytin *a*, so that the concentrations of the two pigments can be inferred by comparing the pre-acid and post-acid absorbance readings (Lorenzen 1967). Chlorophyll *a* and phaeophytin *a* levels were calculated according to Lorenzen (1967), with a correction for phaeopigment content. The equations were as follows:

Chl *a* (mg/g) = $\{([A_{665(a)} - A_{665(b)}] \times 28.66) \times 0.01\}$ / subsample weight (g)

Phaeophytin *a* (mg/g) = $\{([1.72 \times A_{665(b)}) - A_{665(a)}] \times 28.66) \times 0.01$ / subsample weight (g)

Where: $A_{665(a)} = 665a - 750a$

$A_{665(b)} = 666b - 750b$

a = pre-acid reading

b = post-acid reading

For chl *a* analysed from the water samples in Section 3.2.1, 50 ml of water was collected and filtered through a GF/F filter. The water was discarded and the filter was placed into 10 ml of 70% ethanol and extracted as above. However, a Turner Designs Trilogy Fluorometer was used to obtain chl *a* concentrations, rather than a spectrophotometer, because the samples in this experiment contained concentrations of chl *a* that were below the detection limit of the spectrophotometer. The calculation used for the fluorometric method was:

$$\text{Chl a}_{\text{ethanol}}\,(\text{ug/L}) = 0.00219 \times (\text{fluorescence}_{\text{pre-acid}} - \text{fluorescence}_{\text{post-acid}}) \times \text{dilution}$$

Tidal flow through system used for laboratory investigations of benthic microalgae

A tidal flow through system was constructed in the laboratory in an attempt to mimic the conditions in the AHE (Figure 2). The sediment cores (see Sections 3.2.1 and 4.2.1 for core collection method) were placed within 36 plastic containers (12.5 cm diameter, 11 cm tall; Figure 2).

Figure 2: The tidal flow through system used for laboratory investigations of benthic microalgae.

Incoming water (see Sections 3.2.1 and 4.2.1 for filtering method) was supplied to each plastic container and sediment core after being pumped (~ 200 litres per hour by a Bianco Water Feature Pump) from a ten litre bucket underneath the table (Figure 2). Water being pumped from the bucket first entered into a 12 mm internal diameter hose, and then into one of twelve PVC manifolds on the side of the table, which split the water into three separate PVC aquarium tubes (5 mm internal diameter), each leading into the bottom of a separate plastic container (Figure 3a, b). Water in the containers was drained into one of twelve ten litre buckets underneath the table (Figure 2) by another PVC aquarium tube (8 mm internal diameter and 10 cm tall), when it reached the top of the PVC tube (Figure 3). Each bucket and manifold was associated with three plastic containers, which were used collectively as one replicate. Every tube, plastic container, hose and bucket was colour coded to make it easier to decipher which equipment was feeding each sample (Figures 2, 3a). Light was supplied by Philips HPI-T 400 w lights with a diffuser placed underneath, providing a light intensity of 200-300 µmol m^{-2} s^{-1}, depending on the location on the table. White PVC sheets surrounded the experimental setup to ensure that light did not escape (Figure 2). Light cycles and tidal cycles were programmed depending on the time of year and followed ambient patterns for the AHE (see Sections 3.2.1 and 4.2.1 for cycles). Four Hobo® data loggers were used to gather information on temperature and light intensities within the laboratory setup.

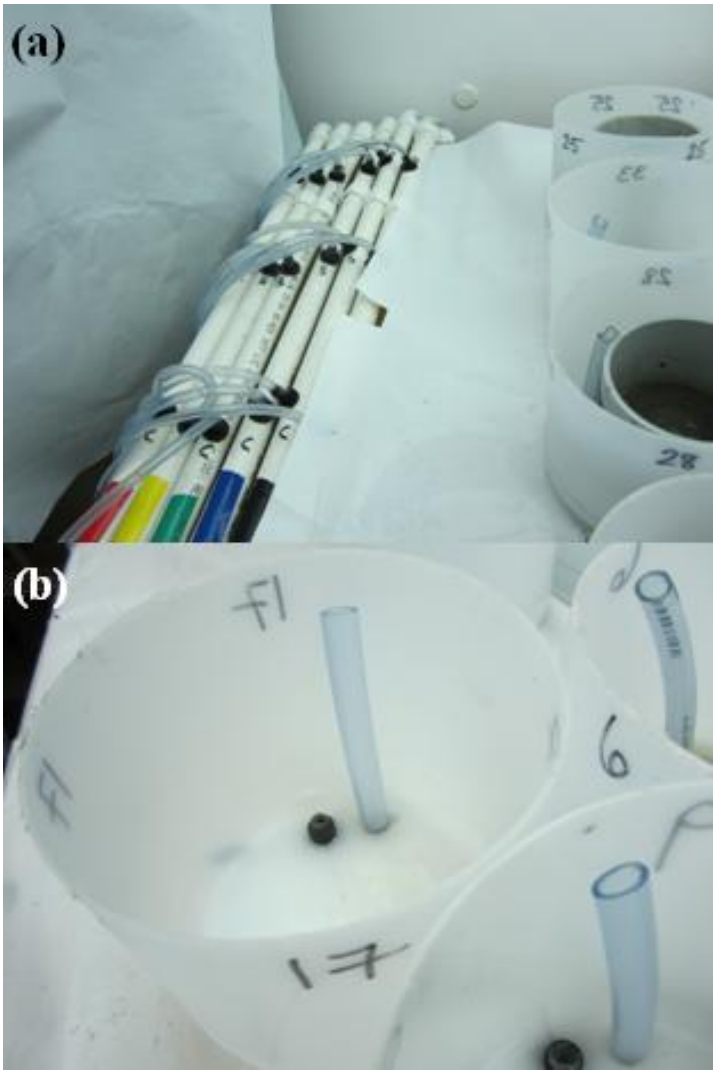

Figure 3: Close up views of portions of the tidal flow through system showing (a) PVC manifolds used to split incoming water into (b) the bottom of three plastic containers without sediment cores. The drain pipes in the plastic containers can also be seen in (b).

Scraping and seeding of benthic microalgae on the sediment surface of experimental treatments

Benthic microalgae are motile and migrate vertically depending on the local environmental conditions (Pomeroy 1959). It is possible to select a time when most BMA are on the surface of the sediment and to remove them almost entirely (Pomeroy 1959). Cores for all laboratory experiments were collected during early afternoon low tides (see Sections 3.2.1, 4.2.1 for coring method), when most BMA were actively photosynthesizing at the sediment surface

(Palmer & Round 1965). To ensure removal of the majority of the BMA mat (Pomeroy 1959, Pinckney et al. 2003), the top centimetre of each sediment core was removed with a knife immediately after cores were transported to the laboratory. All removed sediments were placed into a plastic jar containing one litre of filtered estuary water. The outside of the PVC corers containing the sediment were wiped clean using distilled water and sediments were reinfused with microalgae. The plastic container holding the scraped sediment and seawater was shaken for ten seconds and allowed to settle for three minutes, until large sediment grains were no longer suspended in the water. To ensure that all cores had a similar starting biomass of BMA, five millilitres of water was pipetted from 2 cm below the water surface and placed evenly on each experimental core. The cores were then placed randomly into the tidal flow through system, without water, and the lights were left on for 30 hours to allow the newly added microalgae to grow. Microalgae can completely cover a cleared surface within 48 hours (Owen 1992), so it was believed that 30 hours would be enough time to ensure that cores had a sufficient starting biomass of BMA. The tidal and light regimes were then initiated and water was allowed to flow into the cores. Each PVC corer contained four 3.7 mm diameter holes in its side, approximately 4.5 cm from the bottom. This allowed water from the flow through system to enter the core slowly as the water rose, rather than by spilling over the top of the core and disturbing the sediment.